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Pathophysiology of infections by the gastric trichostrongylid *Obeliscoides* in a rabbit model system

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PATHOPHYSIOLOGY OF INFECTIONS BY THE GASTRIC TRICHOSTRONGYLID *OBELISCOIDES* IN A RABBIT MODEL SYSTEM

A

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PATHOPHYSIOLOGY OF INFECTIONS BY THE GASTRIC TRICHOSTRONGYLID

OBELISCOIDES IN A RABBIT MODEL SYSTEM

by

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ABSTRACT

The gastric trichostrongylid parasite Obeliscoides sp. was isolated from Alaskan snowshoe hares (Lepus americanus) and passaged 3 times in laboratory rabbits (Oryctolagus cuniculus). Despite its low fertility, the isolate persisted, often as occult infections, for up to 45 weeks and produced physiologic effects in clinically normal rabbits. Prominent eosinophilic and hyperplastic lesions of the gastric mucosa occurred during post-inoculation weeks (PIW) 2-15, while mononuclear aggregations were seen in older infections. Gastric lesion severity was directly related to size of the Obeliscoides population, which declined over time and was smaller in secondary infections.

Anorexia occurred within 3 weeks of infective larval inoculation in 12 (of 21) primary and 2 (of 10) secondary infections. Serum total protein, albumin, and the A/G ratio were significantly reduced in anorectic infected rabbits compared to fasted uninfected rabbits. Fecal N excretion was significantly increased between PIW 1 and 5 in rabbits with primary infections, and during PIW 1 and 2 for those with secondary infections. Nitrogen absorption was enhanced during PIW 5-15 of primary infection. Serum gastrin concentrations, determined for the first time in Obeliscoides-infected rabbits by radioimmunoassay, were significantly elevated in primary infections during PIW 6 and 7, while hypokalemia was apparent during PIW 5. Hypermagnesemia occurred in both primary and secondary infections between PIW 8 and 15. Other serum constituents and concentrations of N, Ca and P in the gastrointestinal tract and feces remained largely unchanged.

Total mean retention time (TMRT), 31.8 h, and GI turnover time (GITT), 26.3 h, of the fiber component (determined with Ce-141-marked fiber >355 microns) were significantly prolonged in secondary infections during PIW 16 to 26. TMRT (53.0 h) and GITT (57.0 h) of the liquid component (using Cr-51 EDTA), were determined for the first time in rabbits, and were not significantly changed by Obeliscoides infection.

Persisting populations of this Obeliscoides isolate caused physiologic and pathologic alterations in clinically healthy rabbits. Because these effects were similar to those seen in ruminant Ostertagia spp. infections, this laboratory model could be useful in understanding the pathophysiology of costly production losses that occur in parasitized commercial livestock.

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INTRODUCTION

Hair-like nematodes of the family Trichostrongylidae are among the most common parasites of the gastrointestinal (GI) tract of herbivores. Although various trichostrongylid genera are widely distributed in ruminants and lagomorphs, they are not readily apparent on casual examination but must be found by tedious methods involving washing and sieving of the voluminous ingesta and digestion of the GI mucosal lining. These parasites cause overt disease in young or debilitated animals, but in most adult hosts the trichostrongylid population is small, and clinical signs of their presence are absent.

Recently, it has become apparent that even small trichostrongylid populations may not be benign to their apparently healthy hosts. This is suggested by treatment with anthelmintics, which remove trichostrongylids and increase feed conversion efficiency and meat, milk and wool output in intensive livestock production systems. Even in clinically normal adult hosts considered resistant because few or no trichostrongylid eggs appeared in their feces, anthelmintic usage often yields profitable results (Brunsdon et al, 1986; Symons, 1989; Ploeger, Kloosterman and Borgsteede, 1990b).

Trichostrongylids exert physiologically complex effects on their hosts. In their rapidly growing prepatent and early patent phases, they cause anorexia and diarrhea, especially in immature, naive or stressed herbivores. Since both stress and enhanced parasite transmission frequently accompany confinement, winter housing, or other crowded conditions, trichostrongylids can produce clinical disease and have immediate impacts on livestock production. However, it is the chronic physiological alterations associated with subclinical infections that probably have greater long-term impact on the host population. Trichostrongylids subtly alter a herbivore's absorption and utilization of proteins and certain minerals by affecting not only the morphology and operation of its GI tract, but also its systemic nutrient assimilation. Direct parasitic effects on host GI structure, regional pH, intestinal transit rate and excretion of nutrient from the tract are accompanied by indirect, systemic effects apparent initially as alterations in serum concentrations of proteins, minerals and hormones, and ultimately as production losses (Holmes, 1985; Symons, 1989).

Trichostrongylids may also exert unrecognized effects on wildlife populations by subtly

reducing their hosts' utilization of vital nutrients where availability is limited by seasonal scarcity, habitat loss or rapidly expanding host populations. Early investigators suggested that gastric trichostrongylids could be the direct cause of abrupt declines in their wild host population (Dodds and Mackiewicz, 1961). The lack of correlation between trichostrongylids (number of adult nematodes found in stomachs, or eggs in feces) and host condition or mortality in a number of wild herbivore populations caused this idea to fall into disfavor (Leader-Williams, 1980; Huot and Beaulieu, 1985; Keith et al, 1985, 1986). However, many wildlife investigators fail to search for immature parasite stages within the gastric mucosa and to consider the potential nutritional and reproductive impact of chronic infections (Bye and Halvorsen, 1983; Shaw and Moss, 1989; Shaw, 1990).

To understand the complex physiological effects trichostrongylids exert on both commercial ruminants and wild herbivores, laboratory animals provide efficient, economical model systems (Steel and Symons, 1982; Court et al, 1988; Wagland et al, 1989). Rabbits infected with the natural lagomorph parasite Obeliscoides have been suggested as models for ruminant gastric trichostrongylids, particularly for the closely related genus Ostertagia (Worley, 1963; Russell et al, 1966, 1970; Sollod et al, 1966; Pace and Frandsen, 1982) but this model system has not yet been fully exploited. The recent isolation of Obeliscoides sp. from Alaskan snowshoe hares, Lepus americanus, and its successful passage in standard laboratory rabbits Oryctolagus cuniculus, provided an opportunity to investigate the pathophysiology of gastric trichostrongylids.

The present thesis begins with a chapter describing characteristics of the Alaskan Obeliscoides isolate and the direct effects this parasite exerted on the GI tract of its rabbit host, including clinical signs and necropsy findings. Because the isolate exhibited a pronounced tendency to delay maturation and to persist for up to 45 weeks, physiologic observations could be made on infected hosts in parallel with the development and senescence of their parasite populations over an extended period.

The following 3 chapters focus on various aspects of the physiology of infected hosts over the extended period of parasitism. Potential systemic effects on nutrient assimilation were examined by serial serum collections. Chapter 2 explores changes observed in important, frequently measured serum constituents during the entire infection period, and discusses their implications. Chapter 3 addresses changes in serum concentrations of the GI-related polypeptide hormone gastrin, which has

not previously been evaluated in trichostrongylid-infected rabbits although changes in its concentration are common in parasitized ruminants. Finally, Chapter 4 discusses the gastroenterological effects of Obeliscoides infections, including infection-associated alterations in the fecal excretion of organic and mineral nutrients, changes in nutrient distribution along the tract, and differences in the rate at which ingesta pass through the tract. The single-dose, nonabsorbable marker technique was used to determine passage rates for both the liquid and fiber components of the ingesta. Residence times for the liquid component are reported for the first time in rabbits.

The relevance of the Obeliscoides/rabbit model system is discussed with respect to the pathophysiology of ruminant Ostertagia infections within each chapter. Finally, the significance and unique features of the present model system are discussed, including its utility for understanding the complex effects trichostrongylids exert on both commercial ruminants and wild herbivores.

CHAPTER I

AN INHIBITION-PRONE ISOLATE OF OBELISCOIDES: ESTABLISHMENT AND PASSAGE IN LABORATORY RABBITS

INTRODUCTION

The pathophysiological effects of nematodes parasitic in large ruminants can often be evaluated economically by the establishment and passage of these parasites in laboratory animals. Various nematodes of the genus Trichostrongylus have not only been passaged in small laboratory hosts for over 30 years (Lyons et al, 1987; Savin et al, 1990), but have proved valuable in assaying the potency of ruminant anthelmintics (Court et al, 1988). However, parasites of the important ruminant trichostrongylid genus Ostertagia have yet to find suitable laboratory hosts. Infective larvae, artificially exsheathed larvae, and even transplanted adults of this genus largely fail to reach normal maturity in laboratory species (Wood and Hansen, 1960; Zebrowska-Plata, 1980; Snider et al, 1985; Court et al, 1988). Therefore, the related genus Obeliscoides has been proposed as a model for the study of ostertagiasis in cattle and sheep (Worley, 1963; Russell et al, 1966, 1970; Sollod et al, 1968; Sollod and Allen, 1971).

Obeliscoides cuniculi, the "red stomach worm", is a common parasite of wild lagomorphs in North America (Dodds and Mackiewicz, 1961; Bookhout, 1971; Gibbs et al, 1977; Andrews et al, 1980; Measures and Anderson, 1983a,b; Keith et al, 1985, 1986; Boggs et al, 1990). These studies within natural host populations have concentrated on ecological factors, but larval inhibition in relation to observed seasonal variations in Obl. cuniculi abundance has been discussed, as has the possible contributory role of this parasite to the pronounced cyclicity of its host population (Gibbs et al, 1977; Measures and Anderson, 1983b; Keith et al, 1985, 1986). Some investigators have ascribed gross gastric lesions in Lepus americanus (excessive mucus, petechiation, mucosal hemorrhage, ulceration) to larger burdens of Obl. cuniculi (Dodds and Mackiewicz, 1961; Bookhout, 1971; Measures and Anderson, 1983b), but others have failed to report lesions. Although Sylvilagus floridanus is a more

phylogenetically recent host for Obl. cuniculi (Measures and Anderson, 1983a), no gross lesions have been reported despite the recovery of substantial parasite burdens (Andrews et al, 1980; Measures and Anderson, 1983b; Boggs et al, 1990).

The laboratory passage of five Obl. cuniculi isolates have been reported in the literature, aside from 2 poorly documented early studies (Alicata, 1932; Wallace, 1942) described by Measures and Anderson (1983c). The five isolates are described here. (1) The University of Guelph isolate originated from a snowshoe hare in Ontario, Canada, in May, 1966, and has been the most extensively studied (Fernando, 1968; Stockdale et al, 1970; Fernando et al, 1971; Hutchinson et al, 1972; Michel et al, 1975; Watkins, 1982; Measures and Anderson, 1983c; Watkins and Fernando, 1984). (2) An Ohio isolate originated from a cottontail rabbit (Sylvilagus floridanus) in 1959 and was studied in Michigan, Montana and Pennsylvania as well as at the University of Guelph (Worley, 1963; Fox, 1976; possibly also Sollod and Allen, 1971 and Measures and Anderson, 1983c). (3) An undescribed isolate, possibly one of the two above, was studied at the University of California at Davis (Russell et al, 1966, 1970). (4) The undescribed Alabama isolate, probably originated from a cottontail rabbit, was used for nutritional study at Auburn University (Pace and Frandsen, 1982). (5) An isolate was investigated at the University of Warsaw, Poland, the origin of which was not reported (Helal, Sinski and Bezubik 1987; Helal, Wedrychowicz, et al, 1987; Wedrychowicz et al, 1988). All of these laboratory passages have been in rabbits, Oryctolagus cuniculus.

A few investigators have used Obeliscoides-infected laboratory rabbits to evaluate nutritional effects or anthelmintics (Worley and Thompson, 1963; Watkins et al, 1984; Pace and Frandsen, 1982), but this model system has yet to fulfill its early promise for solving Ostertagia-related livestock problems. Additional isolation attempts seem likely because Obl. cuniculi is one of the most common parasites of widely distributed natural hosts, but these trials are unlikely to reach the literature if they were unsuccessful. The inference might be drawn that Obl. cuniculi isolates have proved unstable or difficult to maintain in laboratory rabbits.

Genetic alterations of the laboratory-adapted Guelph strain of Obl. cuniculi after 16 years of passage have been described (Watkins and Fernando, 1984). Other studies of this nematode in laboratory rabbits have concentrated on the parasite's biology, including the mucosal developmental sequence (Worley, 1963; Russell et al, 1966; Sollod et al, 1968), the delay in larval development induced by environmental preconditioning (Stockdale et al, 1970; Fernando et al, 1971; Hutchinson

et al, 1972; Watkins and Fernando, 1984), and facets of the host immune response (Sollod and Allen, 1971; Michel et al, 1975; Fox, 1976; Helal, Wedrychowicz et al, 1987; Wedrychowicz et al, 1988).

Other facets of Obl. cuniculi infections of laboratory rabbit have not been adequately examined. Details on the initial laboratory passages of the four reported isolates are few (Watkins, 1982). The clinical signs and gross lesions accompanying infections have been described by relatively few investigators, since the focus of many of the studies has been on the parasite rather than the host (Worley, 1963; Russell et al, 1966; Sollod et al, 1968). A single study concentrated on the nutritional aspects of rabbit infections (Pace and Frandsen, 1982). While gastric lesions were noted to be grossly similar to those of Ostertagia, they have been described histopathologically in only one study which ended at 3 weeks post-inoculation (Russell et al, 1970). Finally, although patent infections have been found to persist beyond 9 months (Worley, 1963) or for up to a year (Watkins and Fernando, 1984), most studies have terminated within 12 weeks post-inoculation (Russell et al, 1966; Sollod et al, 1968; Stockdale et al, 1970; Sollod and Allen, 1971; Fernando et al, 1971; Hutchinson et al, 1972; Michel et al, 1975; Fox, 1976: Measures and Anderson, 1983c; Watkins and Fernando, 1984; Helal, Sinski and Bezubik 1987).

The present study records an attempt to isolate and passage Obeliscoides from snowshoe hares, Lepus americanus, at the northwestern extreme of this host's North American range. The goal of a series of 8 experiments was to obtain a stable isolate for use in evaluating the pathological effects of gastric trichostrongylid infections. Observations on the biology and apparent pathogenicity of the early-passage isolate were recorded, particularly with regard to the delayed maturation, reduced fecundity and persistence of senescent or occult infections that uniquely characterized this isolate. Both primary infections (single larval inoculation) and secondary infections (challenge infections of previously exposed rabbits) were evaluated. Some necropsies were delayed up to 45 weeks post-inoculation, and most necropsies were of hosts with post-patent or never-patent infections. Clinical effects on the host are also described. Various pathophysiologic host effects are reported separately, including changes in serum chemistry constituents (Nielsen, Chap. 2), in serum gastrin concentration (Nielsen, Chap. 3), and in changes of gastrointestinal (GI) tract function, including fecal nutrient excretion, nutrient concentration in GI segments, and passage of ingesta components Nielsen, Chap. 4).

MATERIALS AND METHODS

Animals and housing

Standard random-bred New Zealand White rabbits, 11 males and 11 females, were acquired as weanlings (6 to 7 weeks of age) from a commercial supplier[1] and were habituated to the colony schedule for at least one month prior to larval inoculation. The colony was held on a 12-hour light/ 12-hour dark (7am, 7pm) diurnal cycle in an indoor facility entirely separate from other species. Temperatures were maintained between 18 and 24 degrees C. Rabbits were individually housed in stainless-steel cages with grilled floors, with grill spacing wide enough that formed fecal pellets dropped into a holding tray below the floor. The tray was inaccessible to the occupant rabbit. To further preclude autogenous reinfection, trays were emptied frequently (intervals ranging from hourly to 4 days), and both trays and cage interiors were scrubbed weekly with cleaning solutions.

Colony husbandry

Rabbits were fed a limited quantity (130 - 170 grams, depending on age) of commercial pelleted rabbit chow[2], with crude protein >14%, crude fiber <20%, crude fat >2% and ash <10%. This amount of food was entirely consumed within 20 hours when animals had a normal appetite. If more than one-third of the pelleted ration remained in an individual's feed hopper after 24 hours (i.e. at the next scheduled feeding), the rabbit was considered anorectic. After weighing, the residual chow was discarded, and fresh chow was provided in an amount slightly exceeding that which had been consumed the previous day, until the rabbit's consumption returned to the usual daily level.

During the 20-month course of this study, regular fecal flotations (method described below) were used to detect extraneous parasitism by Eimeria spp. or Passaluris ambiguus, the rabbit pinworm. Of 5 separate shipments of weanling rabbits received, at least one rabbit was shedding coccidial oocysts (Eimeria spp.) on initial examination. All 12 weanlings in these 3 shipments were treated with a oral sulfaquinoxaline (SQXN) in drinking water at a dosage of 0.05 to 0.15 mg/kg BW

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[1]R & R Rabbitry; Stanwood, Wash. 98292

[2]"Complete Rabbit Blend", Purina Mills, Inc.; St. Louis, MO 63166

for 15 to 20 days, and tested repeatedly negative prior to admission to the main colony.

Two of the 11 female rabbits were bred and delivered litter during Experiments 2a and 2b. In both cases kits died within 10 days, despite supplemental feeding, due to apparent maternal inattention.

Culture of infective larvae

Infective third-stage Obeliscoides larvae (L3) were obtained by culturing egg-containing fecal or enteric material at room temperature for 8 to 18 days in a moist vermiculite mixture[3] and extracting viable L3 in a Baermann apparatus for 24 hours. Larvae were introduced into new hosts either immediately after recovery and counting or after holding for variable periods (up to 28 weeks) in gauze-covered shallow containers of water in a 4-degree C refrigerator. The numbers of larvae in inocula were determined by counting at least 2 replicated aliquots of 0.1 to 0.5 ml (measured in a volumetric syringe) allowed to vary less than 5%. Larval suspensions were diluted to yield 100 to 400 L3 per aliquot; only motile, active L3, visualized against a grid at 20X, were included in these counts. Suspensions were than concentrated by 3 minutes of low-speed centrifugation into 2-ml volumes for intubation dosing, or 1 to 2 half-ml volumes for gelatin capsule dosing.

Larval inocula were introduced into unanesthetized, restrained rabbits by oral dosing with 1 or 2 gelatin capsules (size "0") containing 0.5 ml of concentrated larval suspension, or into lightly anesthetized (acepromazine 1 mg/kg BW; ketamine 25 mg/kg BW) rabbits by a gastric tube (size No. 5 French) as 2 ml of larval suspension followed by a 3-ml rinse. All rabbits were dosed between 2 and 5 hours after feeding at the usual time.

Experimental design

Of the 22 colony rabbits, 21 rabbits were inoculated once ("primary infections") and 10 of these were inoculated a second time ("secondary infections"), for a total of 31 experimental inoculations of Obeliscoides infective larvae (Table 1.1). One control rabbit received only a sham (water) inoculation prior to necropsy, while 2 other sham-inoculated rabbits were later inoculated

[3]Terra-lite Vermiculite, W.R. Grace Co.; Cambridge, MA 02140

with larvae. All inoculations were in the context of 8 experiments.

In an series of pilot experiments (Experiment 1 through 6), a number of pre- and post-inoculation factors were varied, often simultaneously, to facilitate passage of relatively infertile Obeliscoides isolates. These factors involved both parasite (testing isolates from 3 different snowshoe hare hosts, changing the time infective larvae were stored in the refrigerator) and host (pre- and/or post-inoculation steroid treatment, administering larval doses via gastric intubation vs. oral capsule, and post-inoculation parturition/lactation). The use of relatively large doses of infective larvae (>30,000 L3) were determined to be the only means of obtaining patent infections.

Experiment 7 included 5 rabbits with primary Obeliscoides infections and 3 rabbits with secondary infections, all established by doses of 30,000 to 40,000 second-passage infective larvae. Experiment 8 consisted of a single rabbit with primary Obeliscoides infection established by 45,000 third-passage infective larvae. Details of each of the 8 experiments (12 subdivisions), including information about the hosts (age, sex, weight, etc), parasites (isolate, passage, number of infective larvae (L3), refrigeration prior to inoculation, etc.), duration, and other factors are included in Appendix A and summarized in Tables I.1.

Samples for pathophysiological studies were collected during pilot experiments as well as during Experiments 7 and 8. Blood for serum chemistry and gastrin studies was collected at necropsy from all rabbits, and from the marginal ear vein or central artery of rabbits in Experiments 1, 7 and 8, as described elsewhere (Nielsen, Chap. 2, 3). GI functional studies, including fecal excretion, segmental nutrient concentration, and passage rate investigations (Nielsen, Chap. 4) occurred during Experiments 1, 3a, 5, 6, 7 and 8.

Steroid treatment

In Experiments 2 and 4, 4 rabbits were treated with intramuscular dexamethasone[4] to determine if patency could be induced by exogenous steroid administration. Immunosuppressant doses appropriate to rabbits were determined with reference to Schuchman (1980), Zebrowska-Plata (1980), Snider et al (1985) and Rafferty and Gray (1987). Daily doses of 0.5 mg/kg BW (SID) were

[4]Dexamethasone 2.0 mg Injection; Vedco, Inc., St Joseph, MO 64504

TABLE 1.1. SUMMARY OF PROTOCOL FOR 8 OBELISCOIDES EXPERIMENTS (divided into 12 subdivisions) IN RABBITS

Protocol	TOTAL, all Experiments (ranges)
1. Experiment	
a. No., subdivision	8 Expt's, as 12 subdivisons
b. inoculation #	31 larval inoculations
c. duration (wks)	2 to 45 weeks
2. Number of rabbits infected	
a. water (control)	3 controls (water)
b. primary infections	21 primary infections
c. secondary infections	10 secondary infections
3. Host age (wks)	
a. at start	10 to 45 weeks
b. at end	12 to 91 weeks
4. Host weight (kg)	
a. at start	1.8 to 4.5 kg
b. at end	1.4 to 5.2 kg
5. Infective Obeliscoides larvae (L3) used	
a. isolate/passage*	3 La, 2 FP, 1 SP, 1 TP
b. primary dose size**	500 to 45,500 larvae
c. secondary dose size**	2600 to 50,400 larvae
d. refrig. time (wks)	none to 28 weeks
c. inoculation method:	tube=7 subdiv's, capsule=6 subdiv's
6. Sulfaquinoxaline, PO	
a. no. treated	13 rabbits in 6 subdiv's
b. tmt. period PID (PIW)	
7. Dexamethasone, IM	
a. no. treated	4 rabbits in 2 subdiv's
b. days pre-inoculation	
c. post-inoc. PID (PIW)	
8. Parturition, lactation	
a. no. affected	2 rabbits in 2 subdiv's
b. lactation (PIW)***	(8-9),(17-26)

* Isolates/passage: infective larvae derived from
La=Lepus americanus (3 individuals)
FP=first passage infection (2 rabbits)
SP=second passage infection, combined from 4 rabbits
TP=third passage infection (1 rabbit)

** Dose size = number of infective larvae (L3) in dose

*** Parturition is counted as "lactation day 1"

given to 2 rabbits in Experiment 2 beginning 13 weeks after inoculation and continuing to necropsy 14 and 28 days later (Appendix A). In Experiment 4, 2 rabbits received daily dexamethasone (0.6 and 1.4 mg/kg) for 7 days prior to inoculation and, at a reduced level (0.3 and 0.7 mg/kg), for 28 days following larval inoculation. Standard dosage reduction and alternate-day administration was used to terminate the treatment (Wilcke and Davis, 1982). Mild polydypsia and polyuria was observed in 2 of the 4 treated rabbits.

Fecal examination and culture

Successful reproduction of Obeliscoides (as well as the presence of extraneous parasitism by Eimeria spp and Passaluris ambiguus) was monitored by fecal flotation examinations for each inoculated rabbit. Examinations were conducted 2 to 3 times per post-inoculation week (PIW) up to at least PIW 7, then weekly until the end of the experiment.

Fresh fecal samples of between 2.5 and 3.5 grams were obtained during the usual afternoon periods of high pellet production following twice-weekly cage cleaning. Fecal samples for flotation were individually weighed, mixed with water, ground and strained through cheesecloth, and centrifuged in duplicate 50-ml tubes at 1200 rpm for 4 minutes. After decanting, the sediment was mixed with Sheather's Sugar Solution (SG 1.27), coverslipped, and allowed to equilibrate overnight. Both coverslips were examined at 40X; the minimal detection level of this technique was 0.4 eggs per gram of feces (epg).

Larger volumes of fresh feces, as needed for the culture of infective larvae, were collected 2 to 5 times per day from a wire mesh screen fitted into the bottom of each cage, designed to prevent wetting or soaking of pellets with urine. (Because preliminary attempts to culture larvae from urine-soaked feces failed, all pellets in contact with urine were discarded.) Over the course of 5 experiments, 8 rabbits were selected for larval culture when more than 30 epg Obeliscoides were present on routine flotation examinations.

The day of experimental inoculation was defined as "post-inoculation day 0" (PID 0). Patency was defined as the presence of Obeliscoides eggs, (measuring 85 to 100 by 50 to 60 microns) in a fecal sample at the stated detection limit (0.4 epg). The prepatent period was the PID of first appearance of eggs in the sample. Length of patency was defined as the interval (days or weeks)

between the first and the last appearance of Obeliscoides eggs within the feces; if negative examinations intervened between positive ones, the patency was considered "discontinuous". Patencies were rated according to their maximal level of egg production as "trace" (0.4 to 9 epg), "low" (10 to 29 epg), "moderate" (30 to 99 epg), and "high" (at or above 100 epg).

Necropsy

Euthanasia was accomplished in 21 rabbits by preliminary anaesthesia for intracardiac blood collection (acepromazine 1 mg/kg, xylazine 5 mg/kg, ketamine 60-75 mg/kg) followed by intracardiac injection of concentrated pentobarbital (approximately 150 mg/kg). A single rabbit died spontaneously, as previously noted, with pulmonary lesions. Rabbits were euthanized in the afternoon, between 3 and 4 hours after being offered their daily pelleted ration at the usual time (including all 14 rabbits for which gastric pH was determined) except that food was withheld from 3 rabbits on the day of necropsy and a reduced ration was offered to 2 others. Three of the 22 rabbits were anorectic for at least 24 hours prior to necropsy.

Necropsies of all rabbits included gross examination of the entire gastrointestinal tract (esophagus, stomach, small intestine, cecum, colon), liver and pancreas, as well as the heart, lungs, kidneys, spleen, adrenals, thyroids, reproductive tract, urinary bladder, and major lymph nodes. The central nervous system was not examined. Gastric pH was measured at 2 sites (the mucosal surface of the pyloric antrum and within fluid exuding from contents close to the cardia) within 10 minutes of euthanasia using a compact meter[5] for 13 of the 22 rabbits. Gastric contents were then removed, histopathological sections taken, and for 21 rabbits the remaining (90%) mucosa was digested in room-temperature saline for 24 hours (14 rabbits; Gasbarre, 1987) or in a 37-degree C stirring acid bath (pH 2) for 3 to 7 hours (7 rabbits; Anonymous, 1971). Both gastric contents and fluid from mucosal digests were preserved in 70% ethanol/5% formalin prior to nematode isolation.

Nematode isolation

Total (100%) gastric contents were available from 8 rabbits, while in the remaining 14 rabbits 90% of this material was available for nematode isolation and 10% was reserved for nutrient content

[5]Cardy Compact pH Meter C-1, Horiba Ltd.; Kyoto, Japan

analysis (Nielsen, Chap. 4). Because approximately 10% of the gastric mucosa was preserved for histopathology, 90% was available for digestion from all 22 rabbits.

Preserved contents were sedimented, decanted, brought to working volumes of 250 to 500 ml with water. Contents were thoroughly mixed and between 4 and 6 equal aliquots were taken, representing at least 10% of the working volume (i.e. 9% of the original volume for 14 rabbits), according to the method of Wescott et al (1979). Mucosal digest material was similarly but separately sampled from working volumes of 25 to 250 ml, with between 4 and 13 equal aliquots taken (representing 9 to 60% of the original material). The mean number of adult and immature worms from all aliquots of a working volume were then used to estimate the total content or digest population; standard deviation was determined using the variation among aliquots.

Mature and immature Obeliscoides, as well as mature and degenerating Passalurus, were found and identified to order by searching diluted aliquots at 20X against both light and dark backgrounds. All nematodes were preserved in 70% ethanol/glycerine, and classified as immature vs. adult on the basis of body size and maturity of the reproductive tract (Watkins, 1982; Measures and Anderson, 1983c; Watkins and Fernando, 1984). Identification was not made beyond the generic level for Obeliscoides recovered in this study. "Adult" Obeliscoides were either females 7.0 mm or longer, with fully developed ovijector and patent vulva, considered "sterile" if no developing eggs were present, or males 5.0 mm or longer, with pigmented spicules and well-developed bursa. "Immature" Obeliscoides were smaller than these sizes, usually by a considerable margin, with less developed or inapparent reproductive structures. The "immature" classification included inhibited L4, developing L4, and ensheathed L5 stages.

Histopathological examination

Tissues collected for histopathological examination were placed in 10% neutral buffered formalin, including the cardiac, fundic, antral and pyloric regions of the stomach as well as the duodenum, jejunum, ileocecal region, heart, lung, liver, kidney, and spleen. After processing by routine histological techniques, sections were stained with hematoxylin and eosin. All measurements were made using an ocular micrometer, at 125 or 500x.

Stomachs were described as two histological zones: the fundic or glandular zone

(cardiac/fundus and corpus sections), and the pyloric zone (antral and pyloric sections). All gastric changes in inoculated rabbits were described with reference to sections from the same location in the single uninfected control rabbit, with lesions rated as no change, mild, moderate or severe. Histopathological emphasis was placed on 9 features related to the inflammatory cell population and 8 structural features (Table 1.2).

Total height (or thickness) of the mucosa (1) was measured from the lumenal boundary to the lumenal aspect of the muscularis mucosae. Gastric ridge height was measured from the mucous neck region at the bottom of the gastric pits (the opening of the gastric glands) to the lumen. The mucosal height (1), gastric ridge height (2), and the proportions of total height occupied by gastric ridges vs. glands (2) were measured in those areas of the histologic section where the entire depth of the mucosa lay within the same plane of section. At least 5 separate observations were made to determine the mean height for each section. In intensely hyperplastic areas it was often not possible to follow a single gland within one plane of section, in which case the most representative height over the entire area was taken.

Erosions (4) were excavations in the mucosal surface which did not extend as far as the muscularis mucosae, while ulcerations involved penetration through this boundary into the muscularis mucosae, often with accompanying hemorrhage extending through the surrounding lamina propria and mild to moderate fibrinous exudation into the lumen. "Mucosal hyperplasia" (2) was defined for the fundic zone as the presence of gastric ridges taller than 300 microns, or occupying more than 30% of the total mucosal height in areas where parietal and chief cells were clearly present. For the pyloric zone, it was defined by total mucosal heights exceeding 750 microns, with gastric ridges, including both mucous and undifferentiated epithelial cells occupying more than 70% of the lumenal aspect.

RESULTS

Infective larvae of Obeliscoides were inoculated as 21 primary infections in 8 experiments and subdivisions, with the results presented in Table 1.3. Larvae were also inoculated as 10 secondary infections in 5 experiments and subdivisions, with results presented in Table 1.4.

TABLE 1.2: HISTOPATHOLOGICAL FEATURES EVALUATED FOR GASTRIC SECTIONS FROM RABBITS INOCULATED WITH OBELISCOIDES

(A) Inflammatory cell infiltrate:

1. mononuclear cell numbers increased, as a diffuse infiltrate in the lamina propria of the tunica mucosa
2. mononuclear cell numbers increased, as focal or perivascular accumulations within the tunica mucosa
3. mononuclear cell numbers increased as follicular structures within the tunica mucosa
4. eosinophil numbers increased, as a diffuse infiltrate in the lamina propria of the tunica mucosa
5. eosinophil numbers increased, as focal or perivascular accumulations within the tunica mucosa
6. inflammatory cells disrupting the muscularis mucosae, extending from the deep mucosa into the submucosa
7. submucosa with increased numbers of monocuclear cells
8. submucosa with increased numbers of eosinophils
9. tunica muscularis with increased inflammatory cells

(B) Structural alterations:

1. total height of mucosa (lumen to muscularis mucosae) substantiallly increased or decreased
2. mucosal hyperplasia, proportion of height occupied by gastric ridges increased
3. undifferentiated epithelial cells increased
4. nodular hyperplasia, increased mitotic activity within mucosa
5. cystic dilatation of gastric glands
6. ulcers
7. mucosal erosions, often crater-like
8. mucosal metaplasia (fundus only)

TABLE 1.3. RESULTS OF 21 PRIMARY OBELISCOIDES INOCULATIONS IN RABBITS

1.	Experiment, subdivision	1	2a	3a	3b
2.	No. rabbits inoc'd	3	2	4	1
3.	Fraction anorectic	2/3	0/2*	0/4	0/1
	a. duration (days)	3-5	-	-	-
	b. during (PIW)	(2-3)	-	-	-
4.	Ratio patent:nonpatent	2:1	2:0	2:2	1:0
5.	Prepatent period, to				
	a. PID	14-19	14	14,21	17
	b. (PIW)	(2-3)	(2)	(2,3)	(3)
6.	Patency				
	a. length (weeks)	2-3	1-3	2,20	2
	b. no. weeks >30 epg	none	<1	0,14	none
	c. max epg observed	1,6	2,30	1,478	5
	d. max epg during (PIW)	(3,4)	(3,4)	(4,9)	(4)
7.	Fraction with concurrent				
	a. Eimeria >1.0 opg	0/3	0/2	0/4	0/1
	no. weeks	-	-	-	-
	b. Passalurus > 10 epg	0/3	2/3	1/3	0/1
	no. weeks	-	1-4	1	-
8.	Nonpatent infections observed to (PIW)	(5)	-	(8,16)	-
9.	Patent infections observed after patency ended				
	a. fraction of total	0/2	2/2	2/2	1/1
	b. no. of weeks	-	8,20	2,19	3
10.	Fraction re-inoculated in Expts.	2/3 2b	1/2 5	4/4 4,5,7c	1/1 4
11.	Fraction necropsied	1/3	1/2	0/4	0/1
	a. PID	17	118		
	b. (PIW)	(3)	(17)		

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* Excluding 10 days of anorexia during PIW 16-17 associated with parturition in one individual

** Individual euthanized on PID 12 (PIW 2) due to acute dyspnea

Table 1.3, page 2

1.	Experiment, subdivision	6	7a	7b	8
2.	No. rabbits inoc'd	5	3	2	1
3.	Fraction anorectic	4/5	3/3	2/2	1/1
	a. duration (days)	3-9	8-16	5-13	4-7
	b. during (PIW)	(1-2)	(1-3)	(1-2)	(1)
4.	Ratio patent:nonpatent	4:1	3:0	2:0	0:1
5.	Prepatent period, to				
	a. PID	13-38	15-28	16,48	-
	b. (PIW)	(2-6)	(3-4)	(3,7)	
6.	Patency				
	a. length (weeks)	9-22	8-10	3,7	-
	b. no. weeks >30 epg	2-11	0-2	0-1	
	c. max epg observed	36-243	6-118	13,216	
	d. max epg during (PIW)	(4-10)	(4-10)	(5,11)	
7.	Fraction with concurrent				
	a. Eimeria >1.0 opg	2/5	2/3	2/2	1/1
	no. weeks	1-2	1-2	1-2	1
	b. Passalurus > 10 epg	4/5	2/3	0/2	1/1
	no. weeks	1-4	1		- 2
8.	Nonpatent infections observed to (PIW)	(2)**	-	-	(5)
9.	Patent infections observed after patency ended				
	a. fraction of total	4/4	1/3	0/2	-
	b. no. of weeks	9-18	1	-	-
10.	Fraction re-inoculated in Expts.	2/5 7c	0/3 -	0/2 -	0/1 -
11.	Fraction necropsied	3/5	3/3	2/2	1/1
	a. PID	12**, 259-285	73-101	35,72	35
	b.(PIW)	(2), (37,41)	(11-15)	(5, 11)	(5)

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* Excluding 10 days of anorexia during PIW 16-17 associated with parturition in one individual

** Individual euthanized on PID 12 (PIW 2) due to acute dyspnea

Table 1.3, page 3

		total for all 21 primary infections:
1.	Total experiments	6 experiments (in 8 subdivisions)
2.	No. rabbits inoc'd	21
3.	Fraction anorectic	12/21 *
	a. duration (days)	3-16
	b. during (PIW)	(1-3)
4.	Ratio patent:nonpatent	16:5
5.	Prepatent period, to	
	a. PID	13-48
	b. (PIW)	(2-7)
6.	Patency	
	a. length (weeks)	1-22
	b. no. weeks >30 epg	0-14
	c. max epg observed	478
	d. max epg during (PIW)	(9)
7.	Fraction with concurrent	
	a. Eimeria >1.0 opg	7/21
	no. weeks	1-2
	b. Passalurus > 10 epg	10/21
	no. weeks	1-4
8.	Nonpatent infections observed to (PIW)	(2**,5-16)
9.	Patent infections observed after patency ended	
	a. fraction of total	10/16
	b. no. of weeks	2-20
10.	Fraction re-inoculated in Expts.	10/21 2b, 4, 5, 7c
11.	Fraction necropsied	11/21
	a. PID	12-285
	b. (PIW)	(2-41)

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* Excluding 10 days of anorexia during PIW 16-17 associated with parturition in one individual

** Individual euthanized on PID 12 (PIW 2) due to acute dyspnea

TABLE 1.4. RESULTS OF 10 SECONDARY OBELISCOIDES INOCULATIONS IN RABBITS

1.	Experiment, subdivision	2b	4a	4b	5
2.	No. rabbits inoc'd	2	2	1	2
3.	Previous inoculation				
	a. was Expt.	1	3a,3b	3a	2a,3a
	b. no. weeks from primary to secondary inoculation	5	8	8	16,23
4.	Fraction anorectic	0/2*	2/2	0/1	0/2
	a. duration (days)	-	3-5	-	-
	b. during (PIW)	(4-5)			
5.	Ratio patent:nonpatent	0:2	0:2	0:1	2:0
6.	Prepatent period, to				
	a. PID	-	-	-	27,34
	b. (PIW)				(4,5)
7.	Patency				
	a. length (weeks)	-	-	-	13,15
	b. no. weeks >30 epg				10,13
	c. max epg observed				175,541
	d. max epg during (PIW)				8,10
8.	Fraction with concurrent				
	a. Eimeria >1.0 opg	0/2	0/2	0/1	0/2
	no. weeks	-	-	-	-
	b. Passalurus > 10 epg	2/2	1/2	0/1	0/2
	no. weeks	1	1	-	-
9.	Nonpatent infections observed to (PIW)	(9,15)	(7,13)	(14)	-
10.	Patent infections observed after patency ended				
	a. fraction of total	-	-	-	2/2
	b. no. of weeks				21,26
11.	Necropsied on				
	a. PID	62,99	47,86	91	272,313
	b. (PIW)	(9,15)	(7,13)	(14)	(39,45)

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* Excludes 15 days of anorexia during PIW 7-9 associated with parturition in one individual

Table 1.4, page 2

			total for 10 secondary infections
1.	Total experiments	7c	4 experiments (in 5 subdivisions)
2.	No. rabbits inoc'd	3	10
3.	Previous inoculation		
	a. was Expt.	3a,6	1,2a,3a,3b,6
	b. no. weeks from primary to secondary inoculation	28,44	5-44
4.	Fraction anorectic	2/3	4/10
	a. duration (days)	5-6	3-6
	b. during (PIW)	(1)	(1, 4-5)
5.	Ratio patent:nonpatent	0:3	2:8
6.	Prepatent period, to		
	a. PID	-	27,34
	b. (PIW)		(4,5)
7.	Patency		
	a. length (weeks)	-	13,15
	b. no. weeks >30 epg		10,13
	c. max epg observed		175,541
	d. max epg during PIW		(8,10)
8.	Fraction with concurrent		
	a. Eimeria >1.0 opg	1/3	1/10
	no. weeks	1	0-1
	b. Passalurus > 10 epg	0/3	3/10
	no. weeks	-	0-1
9.	Nonpatent infections observed to (PIW)	(10-14)	(7-15)
10.	Patent infections observed after patency ended		
	a. fraction of total	-	2/2
	b. no. of weeks		21,26
11.	Necropsied on		
	a. PID	65-94	47-313
	b. (PIW)	(10-14)	(7-45)

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* Excludes 15 days of anorexia during PIW 7-9 associated with parturition in one individual

Patency levels suitable for further passage were generated in laboratory rabbits only when doses of more than 15,000 infective larvae of the present Obeliscoides isolate were used. Of 12 rabbits receiving between 500 and 8300 infective larvae (refrigerated from 0 to 15 weeks) in the first 4 experiments, 6 rabbits developed patent infections but in only 1 case did levels exceed 6 epg, and that for only a 4-day period. Accordingly, infective larval dosages were increased levels to between 13,000 and 20,000 L3 in Experiments 3 and 4, and of 5 rabbits inoculated, 3 developed patencies ranging from 36 to 478 epg and lasting for 9 to 20 weeks. The remaining 14 infections (in Experiments 4 through 8) utilized infective larval doses of 29,000 to 50,400.

Initial transfer of the isolate from hares to rabbits

Initial isolates of infective larvae (L3) of Obeliscoides were obtained by culturing the cecal, colonic, and rectal contents as well as the fecal pellets of 3 adult male snowshoe hares (Lepus americanus) obtained in the Fairbanks area during spring, 1987. (Individual hares are identified in Appendix A as "La" followed by a 4-digit number: 3798, 3806, and 3807.) The presence of adult Obeliscoides sp. in the stomach was verified at necropsy, and hares were found to be shedding between 70 and 350 epg of feces (epg) (as well as 1 to 34 epg nematodirids and numerous coccidial oocysts).

Enough hare-origin larvae were available to inoculate 9 laboratory rabbits (twice for 2 rabbits) with doses ranging from 500 to 15,000 L3 in Experiments 1, 2a, 2b, and 3a (Appendix A). Anorexia was observed in only 2 of the 11 infections, and 6 infections developed detectable patency (Table 1.3, 1.4). However, patency exceeded 30 epg in only 2 rabbits (3537 and 8588), both with primary infections (Table 1.3). Fortunately, none of the hare-origin material resulted in the appearance of detectable Eimeria spp. or nematodirids in the recipient rabbits. In the single Pasturella-associated spontaneous death, patent at 5.0 epg, no adult or immature nematodes were present in the stomach content or digest, but 35 adult Obeliscoides, including egg-containing females, were found within the content of the cecum and colon (Table 1.5).

Sources of first-, second- and third-passage isolates

Two first-passage isolates were examined. One of them (3806-88) produced only trace-level patency (Table 1.3) in the single recipient rabbit (Experiment 3b) which received 2800 L3, and was

TABLE 1.5. NECROPSY FINDINGS FOR 11 PRIMARY OBELISCOIDES INFECTIONS IN RABBITS

1.	Experiment, subdivision	1	2a	6	6
2.	Inoculation no.	2	5	18	19
3.	Isolate/passage*	La3798	La3806	FP	FP
4.	Dose (no. L3)	500	2,500	35,200	16,300
5.	Necropsied during (PIW)	(3)	(17)	(2)	(37)
6.	Obeliscoides isolations:				
	a. total (mean estimate)	35**	0	30,800	1,400
	b. SD	30	-	2,400	250
	c. adult nematodes	35	-	0	0
	d. immature nematodes	0	-	30,800	1,400
	e. establishment@	7%	-	87%	9%
7.	Gross gastric lesions:				
	a. plaques, pale areas (none/few/some/many)	none	none	many	some
	b. focal erosions (none/few/some/many)	none	none	many	none
	c. excess mucus?	no	no	yes	no
8.	Histopath. gastric lesions@@ (none/mild/moderate/severe)	mild	none	severe	moderate
9.	Gastric pH at necropsy				
	a. mucosa at antrum	n/a	n/a	n/a	1.3
	b. fluid at cardia	n/a	n/a	n/a	1.7

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* Infective larvae derived from LA=Lepus americanus, FP=first passage infection, SP=second passage infection, TP=third passage infection

** Recovered from caecum/colon in this individual only

@ Establishment: percentage of dose, i.e. total isolates x 100 divided by dose

@@ Histopathologic lesions: characteristics of levels described in text

n/a pH information not available

Table 1.5, page 2

1.	Experiment, subdivision	6	7a	7a	7a
2.	Inoculation no.	22	24	25	23
3.	Isolate/passage*	FP	SP	SP	SP
4.	Dose (no. L3)	30,400	31,300	31,400	29,700
5.	Necropsied during (PIW)	(41)	(11)	(14)	(15)
6.	Obeliscoides isolations:				
	a. total (mean estimate)	25	3,700	1,200	3,700
	b. SD	25	500	700	1,300
	c. adult nematodes	0	500	600	1,400
	d. immature nematodes	25	3,200	600	2,300
	e. establishment*	0.1%	12%	4%	13%
7.	Gross gastric lesions:				
	a. plaques, pale areas (none/few/some/many)	none	many	some	many
	b. focal erosions (none/few/some/many)	none	few	few	few
	c. excess mucus?	no	yes	yes	yes
8.	Histopath. gastric lesions** (none/mild/moderate/severe)	mild	severe	severe	severe
9.	Gastric pH at necropsy				
	a. mucosa at antrum	1.0	1.5	1.1	1.3
	b. fluid at cardia	2.0	4.2	3.2	2.1

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* Infective larvae derived from LA=Lepus americanus, FP=first passage infection, SP=second passage infection, TP=third passage infection

** Recovered from caecum/colon in this individual only

@ Establishment: percentage of dose, i.e. total isolates x 100 divided by dose

@@ Histopathologic lesions: characteristics of levels described in text

n/a pH information not available

Table 1.5, page 3

					total for all 11 primary infections
1.	Experiment, subdivision	7b	7b	8	6 Expt's
2.	Inoculation no.	29	30	31	
3.	Isolate/passage*	SP	SP	TP	La=2, FP=3, SP=5, TP=1
4.	Dose (no. L3)	29,500	30,900	45,000	500 - 45,000
5.	Necropsied during (PIW)	(5)	(11)	(5)	(3-41)
6.	Obeliscoides isolations:				
	a. total (mean estimate)	18,900	700	43,000	0 - 43,000
	b. SD	1,400	200	1,800	
	c. adult nematodes	1,800	550	10	0 - 1,800
	d. immature nematodes	17,100	150	43,000	0 - 43,000
	e. establishment*	61%	2%	96%	0 - 96%
7.	Gross gastric lesions:				
	a. plaques, pale areas (none/few/some/many)	many	few	many	none=3, few=1, some=2, many=5
	b. focal erosions (none/few/some/many)	none	none	some	none=6, few=3, some=1, many=1
	c. excess mucus?	yes	yes	yes	yes=7, no=4
8.	Histopath. gastric lesions**	severe	severe	severe	none=1, mild=2, moderate= 1, severe=7
9.	Gastric pH at necropsy				(n=8)
	a. mucosa at antrum	1.3	1.3	1.0	1.0-1.5
	b. fluid at cardia	2.4	4.4	2.5	1.7-4.4

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* Infective larvae derived from LA=Lepus americanus, FP=first passage infection, SP=second passage infection, TP=third passage infection

** Recovered from caecum/colon in this individual only

@ Establishment: percentage of dose, i.e. total isolates x 100 divided by dose

@@ Histopathologic lesions: characteristics of levels described in text

n/a pH information not available

not passaged further. The second one (3807-37) was initially more prolific and was the source of infective larvae for all further studies. The result of the largest hare-origin dose, eggs from this first-passage isolate were shed at levels exceeding 100 epg for 94 days (13 weeks).

First-passage infective larvae of the most prolific isolate were inoculated into 10 rabbits, including 5 primary inoculations in Experiment 6, and 5 secondary inoculations in Experiments 4a, 4b, and 5. Doses ranged from 8300 to 50,400 L3 and refrigeration storage times ranged widely, from none to 8 weeks (Appendix A). Four recipients, including 2 primary-infection rabbits in Experiment 6 and 2 secondary-infection rabbits in Experiment 5, produced moderate to high levels of eggs during patencies which lasted between 9 and 22 weeks (Tables 1.3 and 1.4).

All second-passage larvae cultured from these 4 rabbits were accumulated over relatively long refrigeration times and were combined to form inocula for the 8 rabbits of Experiment 7. Despite doses of about 42,000 L3, none of the 3 secondary infections (Experiment 7c) became patent (Table 1.4). Doses of about 30,000 L3 produced patency in all 5 of the primary inoculations (Experiments 7a, 7b), but only one rabbit produced enough eggs (more than 90 epg for 7 days) to allow culture of third-passage larvae (Table 1.3). A single rabbit was inoculated with these 45,500 larvae in Experiment 8 and was euthanized prior to patency for histopathological sampling (Table 1.3).

Pilot experiments 2 through 6

In an attempt to enhance Obeliscoides patency, post-inoculation immunosuppressant treatments were used in Experiments 2. Four of the rabbits inoculated with 2500 infective larvae in Experiment 2 had not been patent for 8 to 20 weeks. The 2 males, negative for 8 and 12 weeks, were treated with 2- and 4-week courses of IM dexamethasone beginning at PIW 12. The 2 females were bred during PIW 3 and 13, gave birth during PIW 7 and 17, and lactated for about 10 days. Neither of these treatments resulted in the reappearance of patency. Both females became anorectic within 10 days of parturition. Three of the 4 rabbits were euthanized between 9 and 17 weeks after inoculation, and 0, 11, and 150 Obeliscoides were recovered from the stomach (Table 1.6). About half of the adult female nematodes in the largest infection appeared to contain eggs.

In Experiment 3, the refrigeration storage times of infective larvae were prolonged, the

TABLE 1.6. NECROPSY FINDINGS FOR 10 SECONDARY OBELISCOIDES INFECTIONS IN RABBITS

1.	Experiment, subdivision	2b	2b	4a	4a
2.	Inoculation no.	6	7	13	14
3.	Isolate/passage*	La3807		La3807	FP FP
4.	Dose (no. L3)	2,600	2,600	13,000	8,300
5.	necropsied during (PIW)	(9)	(15)	(7)	(13)
6.	Obeliscoides isolations:				
	a. total (mean estimate)	150	10	2,200	1,500
	b. SD	50	11	600	200
	c. adult nematodes	125	10	0	0
	d. immature nematodes	25	0	2,200	1,500
	e. establishment@	6%	0.4%	17%	15%
7.	Gross gastric lesions:				
	a. plaques, pale areas (none/few/some/many)	few	none	many	many
	b. focal erosions (none/few/some/many)	none	none	some	none
	c. excess mucus?	no	no	no	no
8.	Histopath. gastric lesions@@ (none/mild/moderate/severe)	moderate	none	severe	moderate
9.	Gastric pH at necropsy				
	a. mucosa at antrum	n/a	n/a	n/a	n/a
	b. fluid at cardia	n/a	n/a	n/a	n/a

---

* Infective larvae derived from LA=Lepus americanus, FP=first passage infection, SP=second passage infection, TP=third passage infection

@ Establishment: percentage of dose, i.e. total isolates x 100 divided by dose

@@ Histopathologic lesions: characteristics of levels described in text

n/a pH information not available

Table 1.6, page 2

1.	Experiment, subdivision	4b	5	5	7c
2.	Inoculation no.	15	17	16	28
3.	Isolate/passage*	FP	FP	FP	SP
4.	Dose (no. L3)	19,600	50,400	45,500	42,200
5.	necropsied during (PIW)	(14)	(21)	(45)	(10)
6.	Obeliscoides isolations:				
	a. total (mean estimate)	2,500	6	80	435
	b. SD	400	10	75	100
	c. adult nematodes	0	4	5	10
	d. immature nematodes	2,500	2	75	425
	e. establishment@	12%	0.02%	0.2%	0.1%
7.	Gross gastric lesions:				
	a. plaques, pale areas (none/few/some/many)	some	few	few	few
	b. focal erosions (none/few/some/many)	none	none	few	none
	c. excess mucus?	no	no	no	no
8.	Histopath. gastric lesions@@ (none/mild/moderate/severe)	moderate	mild	moderate	moderate
9.	Gastric pH at necropsy				
	a. mucosa at antrum	n/a	1.1	1.1	1.0
	b. fluid at cardia	n/a	2.9	1.7	3.7

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* Infective larvae derived from LA=Lepus americanus, FP=first passage infection, SP=second passage infection, TP=third passage infection

@ Establishment: percentage of dose, i.e. total isolates x 100 divided by dose

@@ Histopathologic lesions: characteristics of levels described in text

n/a pH information not available

Table 1.6, page 3

				total, all 10 secondary infections
1.	Experiment, subdivision	7c	7c	5 Expt's
2.	Inoculation no.	27	26	
3.	Isolate/passage	SP	SP	La=2, FP=3, SP=3
4.	Dose (no. L3)	41,300	44,300	2,600 - 50,400
5.	necropsied during (PIW)	(11)	(14)	(7 - 45)
6.	Obeliscoides isolations:			
	a. total (mean estimate)	7	10	6 - 2,500
	b. SD	13	12	
	c. adult nematodes	0	0	0 - 125
	d. immature nematodes	7	10	0 - 2,500
	e. establishment@	.02%	.02%	.02 - 17%
7.	Gross gastric lesions:			
	a. plaques, pale areas (none/few/some/many)	none	few	none=2, few=5, some=1, many=2
	b. focal erosions (none/few/some/many)	none	none	none=8, few=1, some=1, many=0
	c. excess mucus?	no	no	yes=0, no=10
8.	Histopath. gastric lesions@@ (none/mild/moderate/severe)	mild	mild	none=1, mild=3, moderate=5, severe=1
9.	Gastric pH at necropsy			(n=5)
	a. mucosa at antrum	1.2	1.4	1.0 - 1.3
	b. fluid at cardia	1.4	3.1	1.4 - 3.7

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* Infective larvae derived from LA=Lepus americanus, FP=first passage infection, SP=second passage infection, TP=third passage infection

@ Establishment: percentage of dose, i.e. total isolates x 100 divided by dose

@@ Histopathologic lesions: characteristics of levels described in text

n/a pH information not available

number of L3 dosed was increased, and recipient rabbits were younger (10 weeks) and smaller (about 2 kg) (Appendix A). Clinical signs of illness were not observed. Of the 5 inoculations, 2 were nonpatent and only the largest dose (15,000 L3) resulted in a productive, 20-week patency (Table 1.3) that was the source of first-passage larvae (isolate 3807-37) for the remaining experiments (Figure 1.1). None of the 5 rabbits were necropsied before re-inoculation in Experiments 4, 5, and 7.

In Experiment 4, both pre- and post-inoculation immunosuppressant treatment was used to determine if productive secondary infections could be established. Three previously-dosed rabbits received 8300 to 13,000 infective first-passage larvae refrigerated less than 3 weeks. Two rabbits received daily IM injections of dexamethasone as noted (Appendix A) both prior to and following inoculation, but neither these nor a third (untreated) rabbit developed patent infections (Table 1.3). Brief anorexia was noted in both dexamethasone treated rabbits beginning more than a week after the cessation of steroid treatment, during PIW 4 and 5, in contrast to all other inoculation-associated anorexias observed in this study, which occurred in the first 3 post-inoculation weeks. At necropsy from 7 and 14 weeks post-inoculation, between 1500 and 2500 Obeliscoides were found in the stomachs of all three rabbits (Table 1.4). All of these trichostrongylids appeared immature or infertile.

In Experiment 5, large doses of infective larvae were used to determine if productive secondary infections could be established. Two rabbits which had previously received 2500 and 3000 hare-origin L3 16 and 23 weeks previously were given 45,500 and 50,400 first-passage L3. More than half of the larvae had been refrigerated for 3 to 6 weeks prior to dosing (Appendix A). In both cases Obeliscoides infections with highly productive patencies, lasting 13 and 15 weeks, resulted (Table 1.3; Figure 1.2). These were the only 2 secondary infections to become patent of the 10 secondary challenge inoculations. They were followed through subsequent nonpatent periods of 21 and 26 weeks. At necropsy 39 and 45 weeks after inoculation, 6 and 80 Obeliscoides, primarily immature nematodes and about 10 adults (nearly all mature males), were found in their stomachs (Table 1.4).

In Experiment 6, larger doses of first-passage infective larvae were refrigerated for 0 to 3, 3 to 6, and 7 to 8 weeks prior to administration. Doses of 16,000, 30,000 and 35,000 first-passage larvae were given to 10-week-old rabbits as primary inoculations. The rabbit receiving one of the largest doses, refrigerated for 3 to 6 weeks, developed acute dyspnea after 9 days of anorexia and

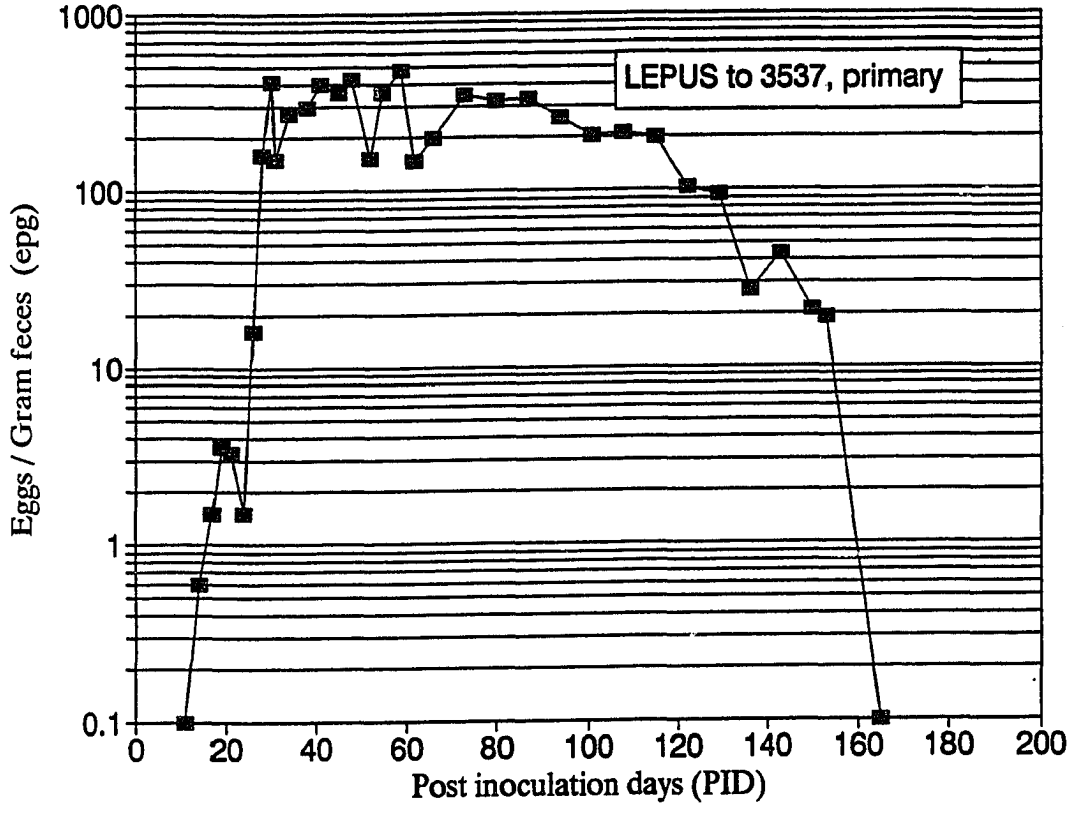

Figure 1.1 Obeliscoides egg production (eggs per gram feces) vs. time since inoculation (post-inoculation day) with 15,000 infective larvae (refrigerated for 8 weeks prior to inoculation) for the most productive isolate of Lepus americanus origin, observed in Experiment 3. These eggs were the source material for the first passage (FP) infective larvae used in Experiments 4, 5, and 6.

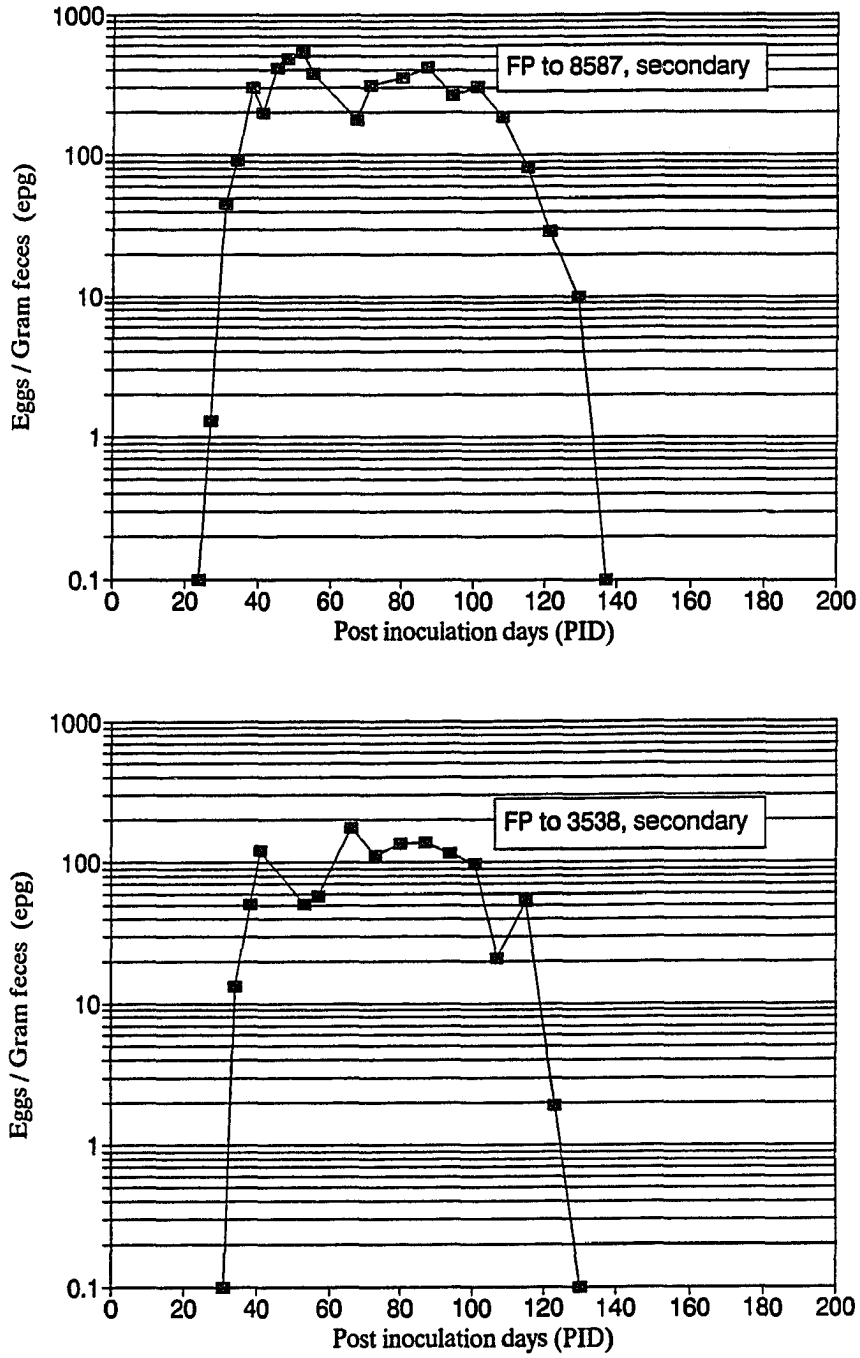

Figure 1.2. Obeliscoides egg production (eggs per gram feces) vs. time since inoculation (post-inoculation day) with 45,500 (top) and 50,400 (bottom) infective first passage (FP) larvae (refrigerated 0 to 6 weeks prior to inoculation), observed in Experiment 5. Both were secondary infections; eggs produced contributed to source material for the second passage (SP) infective larvae used in Experiment 7.

was euthanized on PID 12. Nearly 31,000 immature Obeliscoides were present on the gastric mucosa of this individual (Table 1.4), which also had pulmonary lesions consistent with Pasturella pneumonia.

All 4 of the remaining Experiment 6 rabbits developed patent infections. Larvae refrigerated for less than 3 weeks prior to inoculation generated patencies lasting 9 and 13 weeks (Figure 1.3), while those refrigerated for more than 3 weeks developed patencies lasting 19 and 22 weeks, irrespective of dose size (Table 1.3). Egg output levels in the latter pair of infections tended to be much more variable (Figure 1.4), and that of the rabbit receiving the lower dose (16,000 L3; Figure 1.4, b) was discontinuous (Table 1.3). The latter pair of infections were followed for postpatent periods of 9 and 18 weeks, and when necropsied at 37 and 41 weeks post-inoculation had 24 and 1400 immature Obeliscoides present in their stomachs.

Experiments 7 and 8

Experiment 7 consisted of 5 rabbits with primary infections and 3 with secondary infections. A uniform source of infective second-passage larvae was produced by accumulating larvae from the 4 source rabbits for 7 to 28 weeks, combining them, and dividing the mixture into inocula of about 30,000 L3 for primary infections and about 42,000 L3 for secondary infections (Appendix A). (In the secondary infections, none of the 3 rabbits received larvae cultured directly from their own previous infection.)

All 5 of the primary-group rabbits became briefly anorectic, and all developed patent infections (Figure 1.5), but in only 2 rabbits did these patencies even briefly exceed 20 epg (Table 1.3; Figure 1.6). At necropsy 5 weeks after inoculation, one rabbit had 19,000 Obeliscoides in the stomach, of which nearly 10% appeared to be stunted or infertile adults. The other 4 rabbits were necropsied between 11 and 15 weeks after inoculation, and harbored 700 to 3700 Obeliscoides (Table 1.5). While 13 to 47% of these nematodes were adults, almost all were stunted and infertile.

In contrast, none of the 3 secondary-group rabbits in Experiment 7 developed patent infections, although brief, mild anorexia was observed in 2 hosts (Table 1.4). At necropsy between 10 and 14 weeks after inoculation, 7, 10, and 430 Obeliscoides were found in the stomach (Table 1.6), nearly all immature forms.

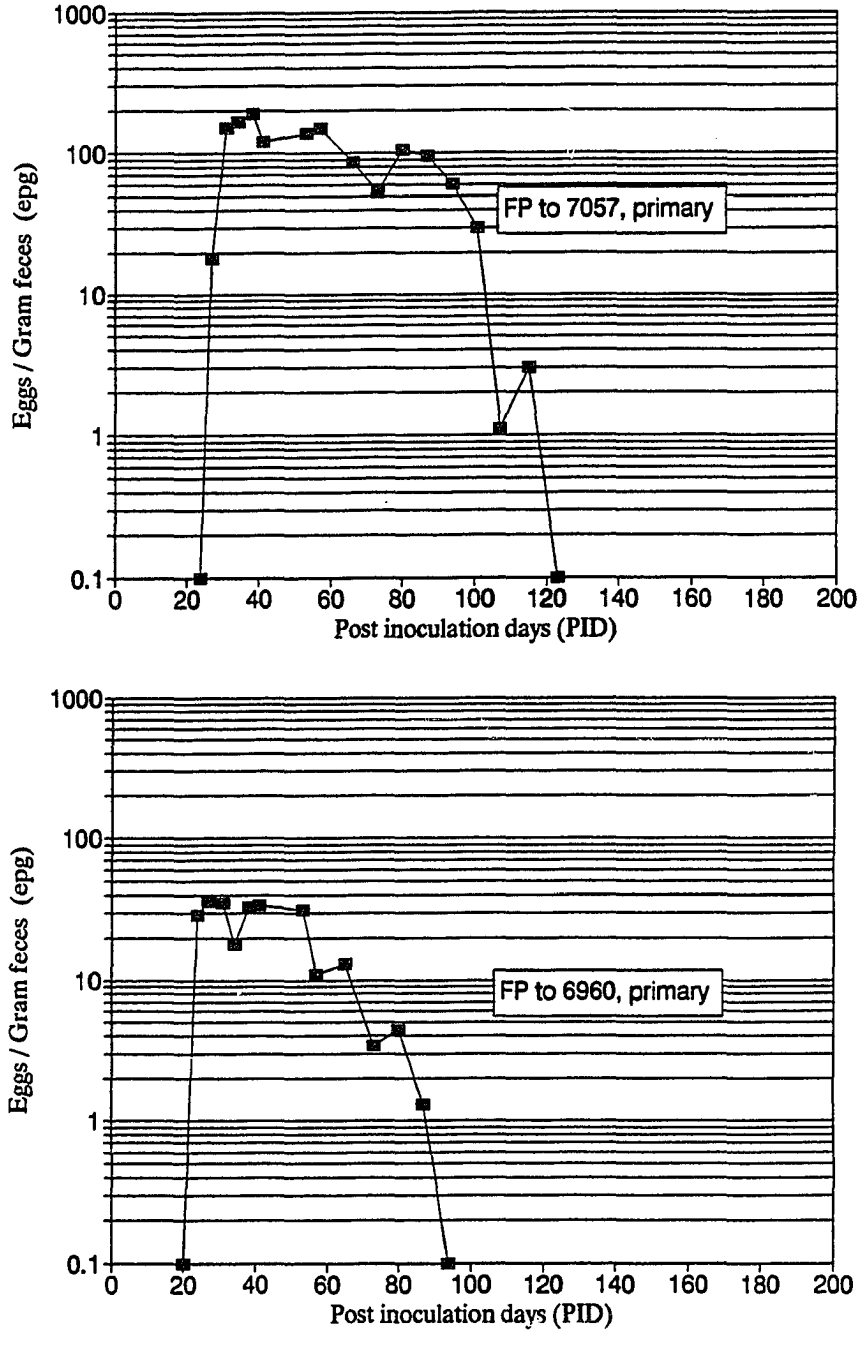

Figure 1.3. First passage (FP) larvae refrigerated for less than 3 weeks (1 to 17 days) prior to inoculation: Obeliscoides egg production (it eggs per gram feces) vs. time since inoculation (post-inoculation day) with (top) 36,000 and (bottom) 16,900 infective FP larvae, observed in Experiment 6. Both were primary infections. Eggs produced by infection (top) contributed to source material for the second passage (SP) infective larvae used in Experiment 7.

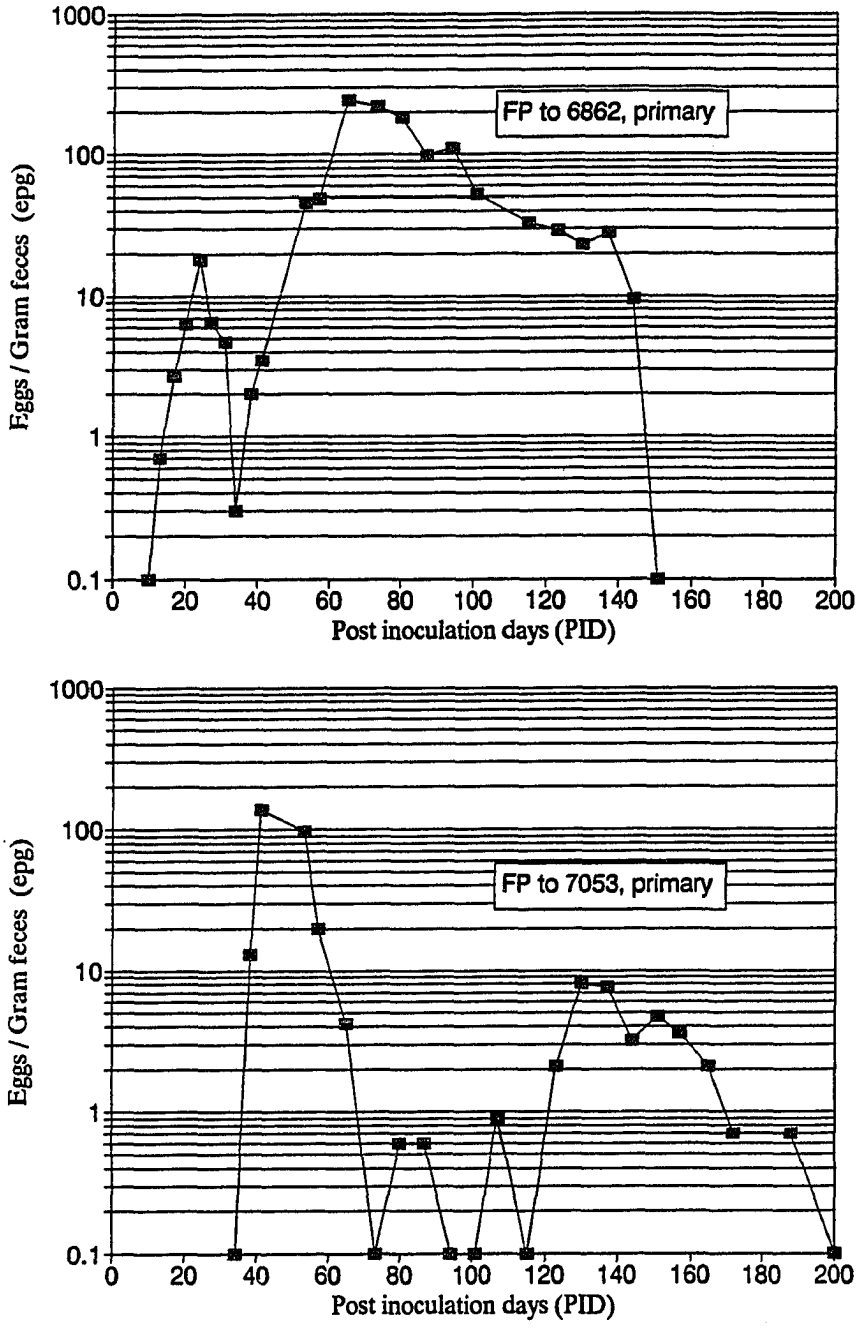

Figure 1.4. First passage (FP) larvae refrigerated for more than 3 weeks prior to inoculation: Obeliscoides egg production (eggs per gram feces) vs. time since inoculation (post-inoculation day) with (top) 30,400 and (bottom) 16,300 infective FP larvae, observed in Experiment 6. FP larvae in (top) were refrigerated 7 to 8 weeks, while larvae in (bottom) were refrigerated 3 to 6 weeks. Both cases were primary infections. Note the long but discontinuous production of infection (bottom). Eggs produced by infection (top) contributed to source material for the second passage (SP) infective larvae used in Experiment 7.

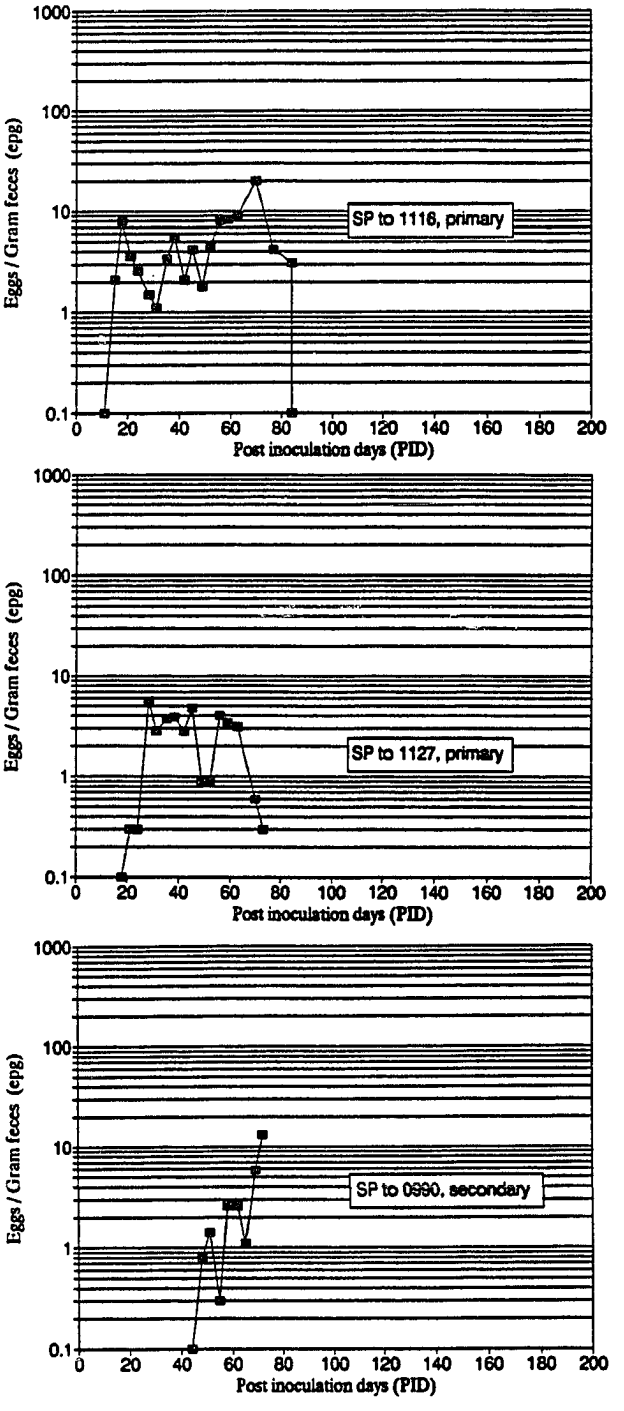

Figure 1.5. Low-level Obeliscoides egg production (eggs per gram feces) vs. time since inoculation (post-inoculation day) with infective second passage (SP) larvae, observed in Experiment 7. Dosages and refrigeration times of SP larvae prior to inoculation were (top) 29,700 L3 refrigerated 9 to 22 weeks, (middle) 31,300 L3 refrigerated 9 to 18 weeks, and (bottom) 30,900 L3 refrigerated 15 to 24 weeks. All three were primary infections.

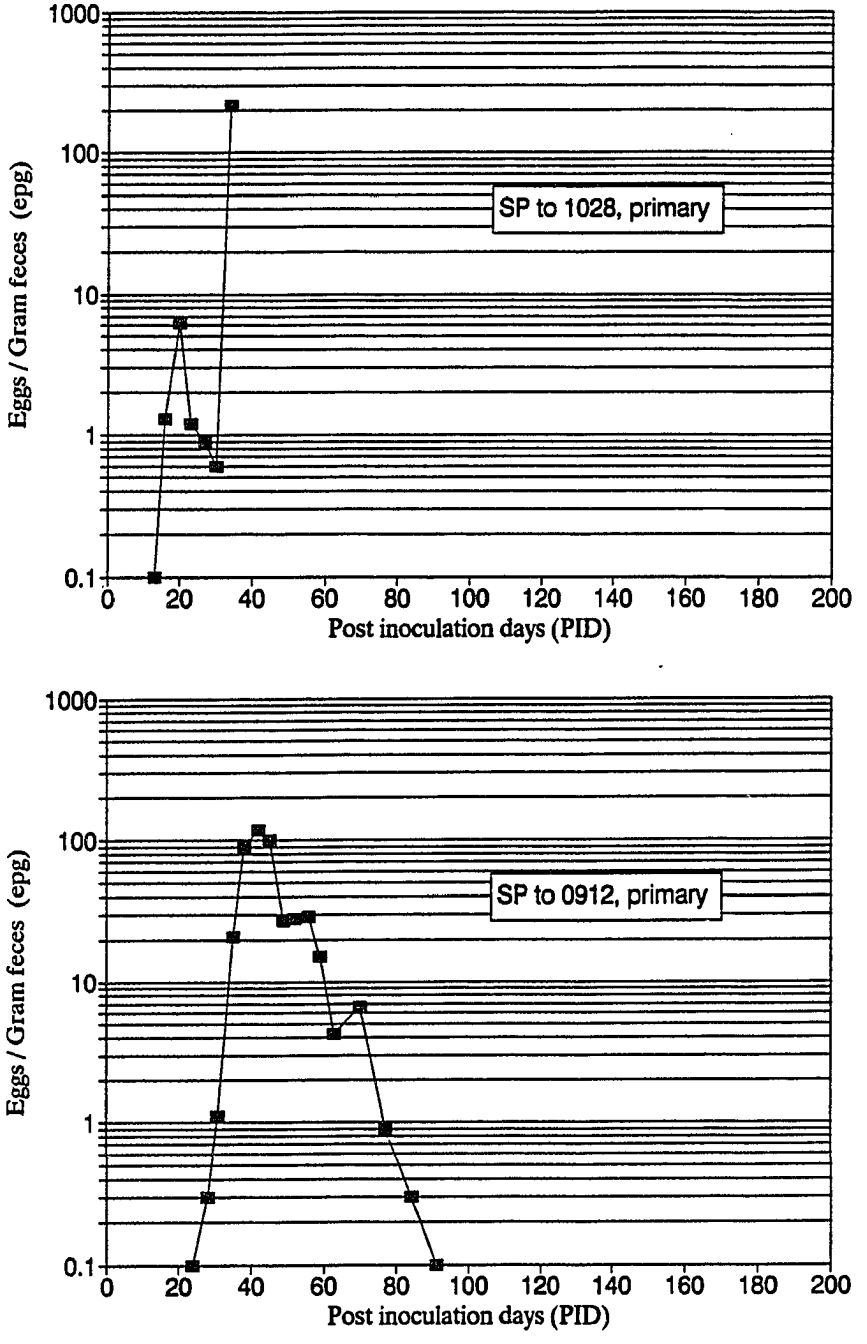

Figure 1.6. Moderate- to high-level Obeliscoides egg production (eggs per gram feces) vs. time since inoculation (post-inoculation day) with infective second passage (SP) larvae, observed in Experiment 7. Dosages and refrigeration times of SP larvae prior to inoculation were (top) 29,500 L3 refrigerated 15 to 28 weeks and (bottom) 31,400 L3 refrigerated 9 to 18 weeks. Both were primary infections. Eggs produced by infection (bottom) were the source of third passage (TP) infective larvae used in Experiment 8.

Experiment 8 was conducted to demonstrate a third passage of the isolate in lab rabbits, and to test the infectivity of briefly-refrigerated larvae that were offspring of a generation subjected to prolonged refrigeration. Third-passage larvae were cultured from a single rabbit in Experiment 7 (Figure 1.6, b), and refrigerated briefly (2 to 10 days) before inoculation as a single large dose (45,500 L3) to a previously uninfected rabbit. This individual was anorectic for 4 days in the first week after inoculation, and did not develop a patent infection prior to necropsy in PIW 5 (Table 1.3). About 43,000 Obeliscoides were present in the stomach at that time (Table 1.5), including an estimated 12 sterile females and no mature males.

Characteristics of patency

Of the 31 inoculations of infective Obeliscoides larvae, 18 resulted in patent infections, including 16 of 21 primary inoculations and 2 of 10 secondary inoculations. Patency length ranged from as brief as 5 days to as long as 20 weeks of continuous egg production. For the 5 infections patent for less than 17 days, maximal egg production levels were all less than 6 epg, while levels for 12 of the 13 infections patent for longer than 19 days were all above 20 epg.

For relatively small doses of snowshoe hare-origin Obeliscoides larvae (Experiments 1, 2, and 3), the prepatent period was consistently between 14 and 21 days (i.e. during PIW 3). Much larger doses of first-passage larvae (Experiments 5 and 6) resulted in more variable prepatent periods, ranging from 13 to 38 days (Table 1.3). Large doses of second-passage larvae (Experiment 7) similarly resulted in prepatent periods between 15 and 28 days, except that one of the five patent infections had a 48-day prepatent period (Figure 1.5, c). Although the later series of inoculations (Experiment 5, 6 and 7) consisted of larger larval dosages (16,000 to 50,000) and were refrigerated from less than 3 weeks to up to 28 weeks, neither dose size nor refrigeration time could be directly associated with shorter or longer prepatent periods.

For 10 rabbits inoculated twice with Obeliscoides infective larvae, the interval between the two inoculations ranged from 33 to 304 days (4 to 44 weeks). Seven of the initial inoculations were of between 1000 and 3000 L3, while 3 were between 15,000 and 36,000 L3. Patent primary infections resulted from 6 of these 10 inoculations. Secondary or "challenge" inoculations were of 2600 L3 (n=2), 8300 to 20,000 (n=3), and 41,300 to 50,400 (n=5). The only two patent secondary infections, both in Experiment 5, had received initial inoculations of 2500 and 3000 hare-origin L3 followed 16

and 23 weeks later by 45,500 and 50,400 first-passage origin L3 (Figure 1.2 a, b).

Isolations at necropsy

Because only 12 of 31 inoculations resulted in levels of Obeliscoides egg production adequate for the culture of infective larvae for further passage, 11 of these infections were allowed to continue to the end of patency and beyond. No infections established by hare-origin isolates or first passage larvae were terminated and necropsied during patency.

Of 21 inoculated rabbits necropsied, including 11 with primary infections and 10 with secondary infections, 16 were examined more than 9 weeks after larval inoculation. Only 4 rabbits had patent infections at the time of euthanasia, with only single infection producing more than 13 epg. The infections of 10 rabbits had never been patent, for periods of from 5 to 17 weeks (excluding the single rabbit euthanized because of dyspnea on PID 12). Infections in the remaining 6 rabbits had ceased their patencies for 2 (n=2) and 11 to 26 weeks (n=4) prior to the time of necropsy.

Even though the opportunities for isolating gastric trichostrongylids from inoculated rabbits was largely delayed into the late-patent or post-patent stages of the infections, Obeliscoides were still recovered from 20 of 21 inoculated rabbits (Table 1.5 and 1.6). Obeliscoides were found in stomachs although host necropsies were delayed as long as 26 weeks after the end of patency. In 9 cases, including 4 primary and 5 secondary infections, only immature nematodes were present at the time of necropsy. In 10 primary infections necropsied between PIW 2 and 15, from 22 to 100% of gastric Obeliscoides present were immature, while of 10 secondary infections necropsied between PIW 7 and 45, from 10 to 100% were immature. While up to 1400 adult Obeliscoides were present in the stomach when primary infections were necropsied (Table 1.5), a maximum of 130 adults were present in secondary infections (Table 1.6).

Fifty mature male Obeliscoides were determined to have the characteristics of the genus (Durette-Desset, 1983; Measures and Anderson, 1983a). While mature male Obeliscoides recovered from source snowshoe hares were 10 to 13 mm in length, those recovered from first, second and third rabbit passages were 5.0 to 7.5 mm, and either stout and robust or thin and somewhat flattened. Similarly, hare-origin mature female Obeliscoides were 18 to 25 mm in length, while rabbit-origin females were generally 7 to 13 mm, often sterile, and usually thin and somewhat flattened. Despite

the presence of a complete ovijector, most of the females that appeared to be adults did not contain eggs within uteri. Developing L4 and ensheathed males and females always exceeded 3.3 mm, while inhibited L4 were always less than this, as previously described (Watkins, 1982; Watkins and Fernando, 1984).

Clinical signs and gross lesions

Depression and moderate to severe anorexia were the primary clinical signs noted in Obeliscoides-infected rabbits. Not only was pelleted ration not consumed in the usual amount, but anorectic rabbits often failed to reingest cecoliths to the full extent. Occasionally, the appearance of dropped cecoliths (recognizable by their distinctive odor) necessitated the daily cleaning of cage floors. The production of regular feces was reduced sharply with the reduced intake. Other clinical signs of infection accompanied anorexia: mild reduction in water consumption, tendency to assume a hunched posture, and, rarely, audible teeth-grinding. Clinical signs were observed following 16 of 31 inoculations (Tables 1.3 and 1.4). Fourteen cases occurred within 3 weeks of larval dosing, but the appearance of signs was delayed (to PIW 4 and 5) until 7 days after cessation of dexamethasone treatment for 2 rabbits in Experiment 4.

Gastric pH, measured at necropsy, was very low on the surface of the fundic mucosa for all rabbits: 1.5 for the control, and between 1.0 and 1.5 for the 13 infected rabbits necropsied between PIW 5 and 45 (Tables 1.5 and 1.6). The pH of fluid near the cardia, associated with the surfaces of food boluses, was considerably more variable: 1.5 for the control, and between 1.4 and 4.4 for the 13 infected rabbits (Tables 1.5 and 1.6). There were no readily apparent relationships between the latter values and larval dose, time post-inoculation, or lesions severity as described below.

On gross examination of the stomach lining, the thin-walled fundic zone, with it slightly textured, deep pink to tan mucosa, could be easily distinguished from the smooth, cream-colored mucosa of the thicker-walled, muscular pyloric zone. The mucosa was marked by mild to severe lesions in 16 of the 21 inoculated rabbits, including 8 of 11 primary infections (Table 1.5) and 8 of 10 secondary infections (Table 1.6).

Three kinds of gross lesions were observed: areas of pale mucosa, focal red or eroded areas, and increased prominence of gastric mucus. Areas of pale mucosa ranged from diffuse, poorly

delineated areas with enhanced surface texture to well-defined grey-white elevations that formed distinct plaques. Plaques were occasionally umbilicated. This type of lesion was encountered only in the fundic zone in 14 rabbits, while in 2 additional rabbits the change extended to include the proximal pyloric canal. Focal red or eroded areas ranged from petechiae within the mucosa to erosions reaching 3 to 4 mm in diameter. Excessive mucus was encountered in 7 of the 11 rabbits from which more than 700 Obeliscoides were later recovered (Tables 1.5 and 1.6). In these cases, a clear, tenacious mucus covered the gastric surface, coating the grossly visible nematodes as they lay with their cephalic ends firmly embedded into the substrate mucosa.

The livers of both rabbits treated with dexamethasone (for 14 and 28 days) up to the time of necropsy (Expt. 2) were obviously enlarged. Only one of these was weighed, and had a liver weight (grams):body weight (kg) ratio of 61.5. The liver:body weight ratios of the two rabbits for which dexamethasone treatment had ceased 28 and 67 days prior to necropsy (Experiment 4) was 27.9 (SD 2.74), not significantly different from those of 12 untreated rabbits (26.8, SD 4.67) (Student's t-test of significance). The liver:body weight ratio of the first rabbit (61.5) differed significantly from those of the 12 untreated rabbits (26.8) at $P<.001$.

Gross pulmonary lesions including purulent bronchitis and exudative pneumonia were apparent in two rabbits. Fibrinous pleuritis accompanied these lesions in one rabbit in Experiment 1, which died spontaneously on PID 17 after 5 days of anorexia. Pasturella multocida was cultured from the lung of this individual. The second rabbit was inoculated in Experiment 6 and euthanized after developing acute dyspnea on PID 12. No other gross lesions were encountered in study rabbits.

Histopathologic findings

Changes in mucosal height (B, 1 in Table 1.2) were determined with reference to the following values for the uninfected control rabbit. In this rabbit, the cardiac zone had a mucosal height of 600 to 900 microns and was relatively narrow. In the fundic zone, total mucosal height was 900 to 1100 microns, with from 10 to 25% of this occupied by gastric ridges. Parietal cells were a prominent component of the well-developed gastric glands; chief cells were less numerous and usually situated slightly deeper in the glands. At the pyloric antrum, height of the normal mucosa dropped somewhat abruptly with the transition to the pyloric gland region, measuring 350 to 450 microns at beginning of the pyloric canal, and gradually increasing to 550 to 650 microns near the duodenal

junction. This region was characterized by a regular dendritic mucosal pattern, with gastric ridges varying from short to tall and occupying from 30 to 80% of the total mucosal height, complementary to the often deep and tortuous gastric pits.

Throughout the gastric mucosa of the control rabbit, inflammatory cells were uncommon. Small groups of mononuclear cells, primarily lymphocytes, with very rare eosinophils, were infrequently distributed in the lamina propria along the lumenal side of the muscularis mucosae. These groups were often perivascular, and never exceeded 70 microns in diameter. Occasional mononuclear cells were apparent in perivascular adventitial areas of the submucosa and muscularis. Neutrophils and globule leukocytes were rare.

Of the 21 rabbits inoculated with infective larvae of Obeliscoides, the histomorphology of 2 individuals, both of which had received dexamethasone for 2 or 4 weeks immediately prior to necropsy (Experiment 2, Appendix A), was essentially identical to that of the control rabbit. The gastric mucosae of the remaining 19 inoculated rabbits, including 3 rabbits with no gross lesions, were characterized by lesions with changes ranging from (1) "severe" for 8 rabbits, and (2) "moderate" for 6 rabbits, to (3) "mild" for 5 rabbits (Tables 1.5 and 1.6). These 3 severity levels, judged on the basis of alterations in the 17 structural and inflammatory features listed in Table 1.2, are described in detail below.

(1) Severe histopathologic lesions were observed in 8 rabbits, all of which had been inoculated with more than 13,000 first-, second- or third-passage larvae. Seven of the 8 rabbits had primary infections, while the eighth had received dexamethasone treatment both prior to and following a secondary larval inoculation (Experiment 4, Appendix A), and had been necropsied 4 weeks after the last treatment. Four of the 8 were necropsied between PIW 2 and 7 weeks, while 4 were necropsied between PIW 10 and 15.

Structural changes altered the entire fundic zone, including areas well away from surface erosions or parasite profiles. Mucosal erosion was apparent in the fundus of all 8 rabbits; frank ulceration was apparent in 2 cases. Hyperplastic change was prominent, including numerous mitotic figures and proliferating mucous and undifferentiated cells forming sessile nodular structures in many areas that had been grossly evident as plaques. The total height of the fundic mucosa increased to range from 1100 to 2000 microns outside of nodular or damaged areas, with gastric ridges occupying

from 30 to 60% of this in many areas. Although structural alterations were less apparent in the pyloric zone, mucosal height increased (to 800 and 1100 microns) in 6 rabbits.

An intense eosinophilic infiltrate characterized the gastric mucosa of all 8 rabbits, particularly in the fundic zone. Eosinophils were prominent not only adjacent to erosive lesions with or without nematode profiles, but also diffusely throughout the tunica mucosa. They were present perivascularly and as dense, suffusive infiltrates along fascial planes of the submucosa of both the fundic and pyloric zones. Eosinophils were also locally prominent within fascia of the tunica muscularis in 4 of the 8 rabbits; blood vessel morphology was normal. Lymphocytes and occasional plasma cells formed confluent layers and small follicular structures (to 60 microns in diameter) in the deep mucosa, while neutrophils and globule leukocytes were rare. Focal collections of degenerating neutrophils, macrophages and eosinophilic debris deep in glands was interpreted as evidence of previous Obeliscoides occupancy. In 7 of the 8 rabbits, nematode profiles (diameters from 25 to 50 microns) were present at all mucosal levels, and were often associated with distended glands lined by disrupted, hyperplastic cells. From 500 to 43,000 Obeliscoides were present in the stomachs of these 8 rabbits at the time of necropsy (Table 1.5).

(2) "Moderate" histopathologic lesions were observed in 6 rabbits, 5 of which had received more than 8300 infective first- or second-passage larvae and were necropsied between 9 and 45 weeks post-inoculation. Five of the 6 infections were secondary. Structural changes in the fundus were erosive rather than hyperplastic, with frequent mucosal excavation ranging from shallow (less than 50% of the total height involved) to deep, crater-like lesions occupying as much as 80% of the mucosal thickness. Cystic dilatation was evident in a few craters. Large areas of normal fundic mucosa were abruptly interspersed with dense mononuclear infiltrates in the deep mucosa, usually at the deep aspect of crater-like lesions. Follicular structures often had diameters exceeding 300 microns, and included both lymphocytes and plasma cells. Mononuclear cells also formed confluent layers along the lumenal aspect of the muscularis mucosae, and frequently extended into the submucosa of the fundic zone. Eosinophils were much less prominent in this group, ranging from diffusely increased to focal accumulations within the lamina propria or perivascular submucosa.

Both erosions and inflammatory cells were infrequent in the pyloric zone, but focal nodular hyperplasia was often prominent. Occasional sessile nodules ranged from 900 to 2300 microns in height and extended for variable distances along the mucosa. Nematode profiles were encountered

in 3 of the 6 rabbits, and nematode-associated debris was seen in a third. Between 80 and 2500 Obeliscoides were recovered from the stomachs of rabbits in this group (Tables 1.5 and 1.6).

(3) "Mild" histopathological lesions were encountered in 5 rabbits, including 3 secondary infections. Four of the 5 were necropsied between PIW 11 and 41. The fifth was the single rabbit dying spontaneously of pneumonia during PIW 3 after a primary inoculation of 500 snowshoe hare-origin infective larvae. Occasional shallow erosions in the fundic zone and rare, localized nodular hyperplasia in the pyloric zone were the primary structural changes encountered in the gastric mucosa of these rabbits. Mononuclear cell numbers were slightly increased over those of the control rabbit, with small follicles ranging from 100 to 200 microns in diameter within the deep mucosa. A few eosinophils were apparent in perivascular areas, primarily in the pyloric zone. There were no nematode profiles within the mucosa of any of the 5 rabbits, although nematode-associated debris was present in a single animal. Between 6 and 25 Obeliscoides were recovered from the stomachs of 4 rabbits (Tables 1.5 and 1.6). While no nematodes were present in the stomach of the fifth rabbit (the pneumonia death), 35 Obeliscoides were found in the cecum and colon (Table 1.5).

All other tissues were histopathologically normal except for the liver and lung of certain rabbits, described here. Massive vacuolar degeneration and hepatocytomegaly was observed in the enlarged livers of both rabbits which had been treated with dexamethasone for 14 and 28 days weeks immediately prior to necropsy. Lesion appearance and distribution was as described and illustrated previously (Fittschen and Bellamy, 1984). Milder hepatocytic changes with evidence of regeneration was apparent in the normal-sized livers of both rabbits necropsied 28 and 67 days weeks after the termination of steroid treatment.

Purulent bronchopneumonia as previously associated with Pasturella multocida infection (Flatt and Dungworth, 1971a, b) was confirmed histopathologically from the two rabbits with gross lesions. While P. multocida infection may occur subclinically (Flatt and Dungworth, 1971a), the only 2 rabbits in the present study with typical respiratory lesions were those with clinical signs of infection. Both individuals were euthanized during the early post-inoculation period (PID 12 and 17). Since both rabbits also had gastric lesions associated with Obeliscoides at necropsy, the infections may have been synergistic. However, in cattle the acute pathogenetic mechanisms of P. haemolytica and Ost. ostertagi infections have been found to differ substantially (Conner et al, 1989).

Concurrent parasitism

The eggs of Passaluris sp. were infrequently present in fecal samples from experimental rabbits. In 10 of 21 primary infections (Table 1.3) and 3 of 10 secondary infections (Table 1.4), more than 10 epg were present at least once during the experimental period. In only one case were eggs present for more than one week. Clinical signs were never associated with the appearance of pinworm eggs. Passaluris sp. were found in the stomach contents of 8 of 22 rabbits at necropsy (Tables 1.5 and 1.6), nearly always in a degenerating, fragmented condition, suggesting that they had been introduced via cecolithic ingestion. As previously observed (Worley, 1963), all gastric pinworms were associated with the content rather than the mucosa, were disintegrating and were clearly nonviable. Pinworms with normal morphology were present in the cecum and anterior colon of all 6 rabbits examined, including all 4 rabbits treated with dexamethasone. Passaluris ambiguus (pinworm) infections are generally considered to be nonpathogenic in rabbits (Wescott, 1974).

Coccidial oocysts were detected in feces at levels exceeding 10 epg in 7 of 21 primary infections (Table 1.3) and in one of 10 secondary infections (Table 1.4). On 2 occasions during Experiment 7, colony-wide oral SQXN treatment was instituted for 15 days when any individual shed more than 10 opg; a single individual was treated during Experiment 8. All patent coccidiosis cases were terminated within a week of detection.

In 6 of the 7 cases, oocysts appeared abruptly in clinically anorectic rabbits within 3 weeks of primary larval Obeliscoides inoculation. Clinical anorexia was attributed to Obeliscoides rather than to Eimeria because most trichostrongylid-infected rabbits that became clinically anorectic failed to shed oocysts or to develop diarrhea, while nearly all pre-infection cases of coccidiosis in weanling rabbits had been accompanied by diarrhea. Neither intestinal nor hepatic coccidiosis were evident histopathologically for any of the study rabbits. While Eimeria spp. are pathogenic in laboratory rabbits, coccidial infections were not considered significant in the present study because continual surveillance and prompt treatment terminated the few cases which occurred, and no signs of coccidiosis were detected at necropsy.

DISCUSSION

Previous reports (Worley, 1963; Russell et al, 1966; Sollod et al, 1968; Fox, 1976; Measures and Anderson, 1983c; Helal, Sinski and Bezubik 1987) attest to the ability of Obeliscoides cuniculi to successfully reproduce in hosts, primarily the laboratory rabbit, that differ at the generic level from its preferred natural hosts. Although the present isolate, which was obtained at the northwestern extreme of the North American range of its natural hosts, shared some biological features with isolates previously studied in rabbits, it was distinguished by its tendency to arrest development in the immature stage irrespective of refrigeration, and its ability to persist for months as an occult infection.

While Pasturella multocida, Passaluris ambiguus, and Eimeria spp. infections occurred within the colony during the study period, their effects were considered to be of negligible significance to the investigation. All 3 agents are common in laboratory rabbits, including the colonies used for previous Obeliscoides and Ostertagia investigations (Worley, 1963; Hutchinson et al, 1972; Michel et al, 1975; Fox, 1976; Watkins, 1982; Measures and Anderson, 1983c; Snider et al, 1985; Helal, Sinski and Bezubik 1987).

Biological and clinical features of Obeliscoides infection

Many of the biological features of the present Obeliscoides isolate are similar to those previously described in laboratory rabbit infections. The length of the prepatent periods was between 13 and 38 days for 17 of 18 patent infections. Other investigators report prepatent periods of 16 to 41 days (Worley, 1963; Russell et al, 1966; Sollod et al, 1968; Fox, 1976; Watkins, 1982; Measures and Anderson, 1983c; Helal, Sinski and Bezubik 1987). Length of continuous patency was from 5 to 139 days (1 to 20 weeks) in the present study, and has previously been reported as 61 to 235 days (9 to 34 weeks) (Worley, 1963; Watkins, 1982; Measures and Anderson, 1983c).

Neither dose size (number of infective larvae in the inoculum) nor the length of time larvae were refrigerated prior to inoculation were directly related to the length of the prepatent period in the present study. (The single exception of 18 patent infections was the extension of the prepatent period to 48 days after 15 to 24 weeks of inoculum refrigeration.) This is in contrast to findings for other isolates, where increasing dose size and/or extending refrigerator storage time (Russell et al,

1966; Helal, Sinski and Bezubik 1987) have increased the length of the prepatent period. Dose size and refrigeration time also did not affect the total length of 11 patencies followed for more than 2 weeks after cessation of egg production, which has not been addressed by previous investigators.

The isolate used in the present study was difficult to establish within standard laboratory rabbits. In the initial experiments, dose sizes of larval inocula were held below 10,000 L3 because previous investigators had reported reduced establishment, egg production levels and shorter patencies for larger doses (Worley, 1963; Russell et al, 1966; Michel et al, 1975). But of 11 rabbit inoculations with from 500 to 15,000 L3 of snowshoe hare origin, only one, the 15,000 L3 dose, resulted in productive patency. Further inoculations indicated this isolate was incapable of generating productive infections at smaller doses, although this characteristic cannot be separated from possible refrigeration effects. In contrast, fertile adult Obl. cuniculi were produced in laboratory rabbits, as well as in hares, cottontails and woodchucks, with initial inoculations of 200 to 500 L3 of snowshoe hare origin (Measures and Anderson, 1983b).

The present Obeliscoides isolate is also distinguished from those previously studied by the low level of its egg production. Maximal production was 541 epg, but only 12 of 31 larval inoculations resulted in patencies above 20 epg for more than 19 days. There were no indications egg production improved with increasing laboratory passage. Egg production levels of other isolates was reportedly much higher: Worley (1963) achieved levels of 5,500 epg with larval dosages of 1000 L3, Watkins (1982) achieved level of 3,100 epg with dosages of 500 L3, and other investigators reached levels of 350 to 1063 epg with larval doses of 500 to 10,000 L3 (Russell et al, 1966; Fox, 1976; Helal, Sinski and Bezubik 1987).

Previous Obl. cuniculi isolates have been maintained in laboratory rabbits passage with routine doses of less than 10,000 L3 for 5 to 16 years (Worley, 1963; Sollod et al, 1968; Sollod and Allen, 1971; Fernando et al, 1971; Fox, 1976; Watkins and Fernando, 1984; Helal, Sinski and Bezubik 1987; Wedrychowicz et al, 1988). Despite early observations on the increased infectivity of bovine-origin Cooperia punctata after one laboratory passage (Wood and Hanson, 1960), there is no evidence that any of these Obeliscoides isolates have adapted to rabbit passage by reductions of required larval dosages. Instead, selection for maximal long-term egg production accompanied by minimal clinical effect seems to have been accomplished, since exposure to more than 10,000 L3 of the Ohio isolate was "not well tolerated" by laboratory rabbits in 1963 (Worley), but after 13 years

of further passage 100,000 to 200,000 L3 of this same isolate were used to generate "immunizing infections" (Fox, 1976).

Because low Obeliscoides egg production made passage of the present isolate difficult, doses of dexamethasone ranging from 0.5 to 1.4 mg/kg were used to induce immunosuppression in rabbit hosts. For rabbits, 0.6 mg/kg BW is a standard therapeutic dose of dexamethasone (Schuchman, 1980), and daily intramuscular doses of from 0.75 to 2.2 mg/kg have been used for immunosuppression (Snider et al, 1985; Rafferty and Gray, 1987). That immunosuppressant levels of dexamethasone were achieved in the present study was verified by hepatomegaly and degenerative hepatocellular lesions previously associated with steroid administration in rabbits (Bhagwat and Ross, 1971; Snider et al, 1985) and dogs (Fittschen and Bellamy, 1984). The liver weight: body weight ratio of one rabbit receiving steroid at the time of necropsy was more than twice that of untreated rabbits, but similar to that of treated dogs (Fittschen and Bellamy, 1984).

Despite this evidence that immunosuppression was achieved in the present study, patency did not appear in any of the four treated rabbits. This is in agreement with previous observations on a variety of trichostrongylid species, and occurred despite a marked reduction of inflammatory cells in gastric lesions. Steroid administration has been successful in suppressing immunity to trichostrongylid infections (Douch et al, 1988; Newlands et al, 1990), preventing the rejection of inhibited larvae (Prichard et al, 1974; Soulsby, 1985), and enhancing maturation (Sollod and Allen, 1971; Behnke and Parish, 1979; Court et al, 1988; Conder et al, 1990). Patency has not appeared, however, in steroid-treated nonpermissive hosts including laboratory rabbits (Zebrowska-Plata, 1980; Snider et al, 1985), perhaps because maturation and fertility are affected by separate immunological mechanisms (Fox, 1976). Both the stage-specifity of local and systemic humoral responses to Obl. cuniculi and, especially, the delayed (to PIW 8 and 13) appearance of these responses have been suggested as a cause of infections' "chronicity" (Wedrychowicz et al, 1988).

For the present isolate, at equivalent (high) doses Obeliscoides fertility declined while clinical effects on the host became more frequent between the first and second laboratory rabbit passages. Anorexia and depression were the primary clinical signs encountered, but scanty fecal production and failure to reingest all cecoliths were also apparent. These signs often accompany the reduced gut motility seen in enteric diseases of rabbits (Cheeke, 1987). The hunched posture of inoculated rabbits is consistent with abdominal discomfort, and is often associated with anorexia in Ostertagia-infected

ruminants (Holmes, 1985). Anorexia, "malaise" and weight loss have been previously related to increasing numbers of infective Obeliscoides larvae in laboratory rabbits (Worley, 1963; Russell et al, 1966), while clinical signs have not been noted in nonpermissive laboratory infections with ruminant Ostertagia (Wood and Hanson, 1960; Zebrowska-Plata, 1980; Snider et al, 1985; Court et al, 1988; Okamoto et al, 1988).

Although host age, dose size and frequency, and strain differences may all influence the appearance and severity of clinical signs in bovine ostertagiasis, anorexia and diarrhea are generally associated with the early patent period (Snider et al, 1981; Symons, 1989). Obeliscoides appears to exert most of its clinical effects slightly earlier than Ostertagia (Russell et al, 1970). In the present study, anorexia associated with primary infections had resolved by PID 17 (for 11 of 12 cases), coinciding with the first appearance of patency between PID 13 and 38. A previous study of large-dose primary Obl. cuniculi infections in rabbits found clinical signs most apparent during PIW 1 and resolving by PIW 2, although infections that did not become patent until PIW 3 to 5 (Russell et al, 1966).

Clinical anorexia in laboratory rabbit hosts is not necessarily associated with high levels of Obeliscoides reproduction. Russell et al (1966) found that increasing larval doses lowered egg production levels despite the exacerbation of clinical signs. Even low-patency primary and nonpatent secondary infections were accompanied by clinical anorexia in the present study. Anorexia was delayed to PIW 4-5 (and appeared 7 days after treatment cessation) only for the 2 rabbits which received steroids pre- and post-inoculation. This suggests that trichostrongylid-associated anorexia may be rooted in a host response which is steroid-susceptible.

Developmental inhibition of in-host Obeliscoides

The ability of the present isolate to persist in rabbits for up to 45 weeks after larval inoculation, often after all external signs of infection had ended, was surprising and unexpected. Evaluation of the actual numbers and maturity of Obeliscoides present within rabbits in the present study was delayed for considerable periods because patent infections were preserved to allow further passage: only 4 of the 21 inoculated rabbits had patent infections at the time of necropsy.

Despite the lack of either clinical signs or patency indicative of infection in 17 of the

necropsied rabbits, 20 of the 21 were found to harbor Obeliscoides. Obeliscoides were recovered from the stomachs of rabbits which had been followed for up to 17 weeks without detectable egg production, or for as long as 26 weeks beyond the end of patency. Immature forms, apparently infertile adults, or both, were recovered from the stomachs of rabbits after inoculation with doses of infective larvae ranging from small (1000 L3) to large (50,400 L3) which had been refrigerated from between 1 day to as long as 28 weeks, or which had never been refrigerated. They were found in association with both primary and secondary infections, including those that were pre-patent, patent, post-patent, or never-patent. Because logistics limited the number of infections observed, statistical analysis of relationships between variables of dose size, refrigeration time, passage number of the isolate, and developmental features of the parasite at necropsy awaits a larger study.

The total number of Obeliscoides recovered at necropsy in the present study was directly related to the amount of time that had passed between inoculation and necropsy, an observation shared by others (Measures and Anderson, 1985c; Helal, Sinski and Bezubik 1987). Percentage establishment (relative to dose) of total Obeliscoides ranged from 0 to 96% for primary infections and from 0.02 to 17% for secondary infections. Other investigators have found establishment percentages ranging from 4 to 30% (Worley, 1963; Russell, 1966; Michel et al, 1975; Measures and Anderson, 1983c), but reported up to 66 and 75% for some isolates under certain conditions (Watkins and Fernando, 1984; Helal, Sinski and Bezubik 1987). These percentages of Obeliscoides recovery have been variously correlated with dose size, refrigeration storage time of the infective larvae, and immunological features of the host (Russell et al, 1966; Michel et al, 1975; Watkins, 1982; Watkins and Fernando, 1984; Helal, Sinski and Bezubik 1987).

Percentage inhibition (of the total Obeliscoides present) has also been correlated with these same factors. For several isolates, refrigeration of infective larvae for 6 to 12 weeks has resulted in maximal inhibition rates of between 10 and 80% (Stockdale et al, 1970; Sollod and Allen, 1971; Fernando et al, 1971; Hutchinson et al, 1972; Watkins and Fernando, 1984; Helal, Sinski and Bezubik 1987). Maximal inhibition rates of 57 to 71% have been achieved with large doses (30,000 to 75,000) of larvae (Russell et al, 1966; Michel et al, 1975), and both dose size and larval refrigeration were held responsible for the 2 to 5% inhibition rate of the Polish isolate (Helal, Sinski and Bezubik 1987). In all of these studies, relative inhibition was determined when rabbits were necropsied between 2 and 8 weeks after primary inoculation. In the present study, primary infections were necropsied between 2 and 41 weeks post-inoculation, and inhibition rates ranged from 22 to 100% of gastric Obeliscoides

irrespective of dose size (ranging from 16,300 to 50,400 L3) and prior refrigeration (ranging from 2 days to 28 weeks).

A variety of host-related factors, including aspects of the immune response, have been associated with larval inhibition and resistance to reinfection in ruminant Ostertagia infections (Michel et al, 1979). Secondary Obeliscoides infections have been investigated only twice previously. Worley (1963) observed that two rabbits with previously patent infections generated by 50 and 160 L3 responded to challenge infections (with 300 L3) with renewed patencies, one of which lasted more than 6 months. Using much larger immunizing infections (75,000 L3), Fox (1976) found that challenge infections (of 5000 L3) resulted in the appearance of stunted and infertile worms at PIW 2, and by PIW 4 100% of the worms remaining were inhibited larvae. In the present study, all 10 rabbits experiencing secondary infections retained Obeliscoides when necropsied between 7 and 45 weeks post-inoculation, although only 2 of these infections had been patent. Between 10 and 100% of these nematodes were immature forms. Sterile adults were found in both secondary infections, as observed by Fox (1976), as well as in primary infections.

Pathology associated with Obeliscoides infections

Of the 21 inoculated rabbits necropsied, 19 had gross or histological lesions apparently associated with Obeliscoides infection. The only 2 rabbits without gastric lesions had received immunosuppressant treatment up to the time of necropsy, suggesting steroid-susceptible mechanisms are important in lesion pathogenesis. Similar findings have been reported for nonpermissive Ost. ostertagi infections, in which lesions were less prominent in rabbits chronically treated with dexamethasone (Snider et al, 1985).

When evaluating the lesions found at necropsy in the present study, three factors must be considered. First, few of the inoculated rabbits were necropsied during patency. No information on gastric pH, numbers of Obeliscoides present in the stomach, or gross and histological changes was available except as determined at the time of necropsy. Increasing gastric pH is a hallmark of ruminant Ostertagia infections and is often associated with clinical anorexia (Symons, 1989). In the present study, there was no apparent difference between the gastric pH of the control rabbit and rabbits inoculated between PIW 5 and 45, although no rabbits were necropsied during PIW 6 and 7, when increased serum gastrin concentrations were found (Nielsen, Chap. 3). Gastric pH of

inoculated rabbits varied considerably, between 1.4 and 4.4. Significant gastric pH increases were observed only on PID 9 and 11 when 100,000 L3 Obl. cuniculi were given as primary infections to rabbits; subsequent gastric pH's were normal (2.3) to PID 22 (Russell et al, 1970). The pH of gastric contents varied widely, between 1.5 and 5.22, for Ost. ostertagi infected rabbits necropsied up to PIW 6 (Snider et al, 1985). Nevertheless, marked gastric lesions were present in the rabbits of both studies.

Second, the passage number of the isolate, the length of time it was refrigerated prior to inoculation, and even the size of the larval inoculum seemed to have no relationship to the severity of lesions observed at necropsy in the present study. Instead, histological lesion severity was directly associated with the number of Obeliscoides actually present in the stomach at the time of necropsy. Association between gross lesion severity (petechiation, ulceration and excessive mucus) and the parasite population size has been previously observed for other Obeliscoides isolates, both in laboratory rabbits (Russell et al, 1966) and in natural hosts (Dodds and Mackiewicz, 1961; Bookhout, 1971; Measures and Anderson, 1983b).

The number of Obeliscoides present at necropsy in this study was in turn related to both the primary vs. secondary nature of the inoculation and the age of the infection. Lesions considered "severe" were apparent in all 7 primary infections necropsied before PIW 15, and were associated with the recovery of 700 to 43,000 total Obeliscoides. Many of the features of type I ruminant Ostertagia spp infections (Ritchie et al, 1966; Armour et al, 1966; Poynter, 1966; Snider et al, 1981; Blanchard and Wescott, 1986) were apparent in these lesions, including both inflammatory alterations (intense eosinophilic infiltration, often with extension below the muscularis mucosae, and focal and diffuse mononuclear cells at all mucosal levels) and structural changes (focal erosions and ulcerations, plaque formation, the de-differentiation of glandular elements, nodular hyperplasia of mucous-producing cells, increased mucosal thickness). Globule leukocytes were the only elements of ruminant Ostertagia lesions not observed in the present study. Although special fixatives or stains may be necessary to distinguish this type of leukocyte from among the large-granuled eosinophils characteristic of rabbits (Miller et al, 1967), previous descriptions of both Ostertagia- and Obeliscoides-induced lesions in rabbits and gerbils also failed to find this cell type (Snider et al, 1985; Court et al, 1988).

Third, the tendency of the present strain to delay or avoid maturation is an important factor in understanding the nature of the lesions associated with it. Lesions considered "moderate" occurred

in secondary infections necropsied between PIW 9 and 45, from which 80 to 2500 Obeliscoides were recovered. In these lesions, large mononuclear follicles of a presumably reactive nature were prominent in the fundus while nodular hyperplasia dominated the pyloric region. Even when fewer than 35 Obeliscoides were recovered at the necropsy of 41-week-old infections, lesions of "mild" severity were distinguishable from the uninfected control by mucosal lymphoid aggregations, as well as by fundic erosions and focal nodular hyperplasia.

Although the histopathologic changes observed in the present study shared many features with those described by Russell et al (1970), their presence was considerably prolonged compared to the time frames reported in that study. There, the most prominent mucosal lesions occurred within the first 2 weeks following the inoculation of 100,000 L3 as primary infections; glandular epithelium was well-differentiated, eosinophils were scattered, and lymphocytic follicles were observed during PIW 3, in association with the maturation of most of the Obl. cuniculi by that time (Russell et al, 1970). In contrast, intense eosinophilic mucosal infiltration was apparent up to PIW 15 in the present study, and mononuclear follicles were only prominent after PIW 10, coinciding with the tendency of the isolate to delay maturation.

Both the number of trichostrongylids present and the age of the infection are important determinants of lesion characteristics in ruminant Ostertagia spp infections. The prominence of lymphoid follicles and hyperplastic change in persisting Obeliscoides infections in the present study is similar to lesions described for ruminants 9 or more weeks after single inoculations of Ostertagia spp. (Armour et al, 1966; Snider et al, 1981; Blanchard and Wescott, 1986). However, different isolates of this widely distributed genus may vary considerably in their pathogenicity, based in part on their innate tendency to arrest development.

For ruminants, Ostertagia-induced lesions have characteristics determined not only by the direct (type I) vs. latent (inhibited, pre-type II) nature of the infection (Poynter et al, 1966; Snider et al, 1981; Symons, 1989), but also by the tendency of particular strains to inhibit in response to environmental stimuli, irrespective of the actual presence of the stimuli (Smeal,1982). The so-called "inhibiting strains" of Ostertagia provoke a more vigorous cellular and antibody response, manifested histologically as enhanced numbers of eosinophils, globule leukocytes, and focal lymphoid aggregations (Smeal, 1982).

Mucosal mononuclear aggregations become more prominent and larger after PIW 10 (Blanchard and Wescott, 1986) and in chronic, pre-type II Ostertagia infections (Snider et al, 1981, 1983). Lymphoid accumulations within the deep mucosa have been considered characteristic of pre-type II ostertagiasis in previously exposed hosts, and are associated with the development of an immunity differentially affecting developing nematodes more than arrested, inactive larvae (Snider et al, 1981).

In addition to these inflammatory alterations, structural changes have also been associated with chronic Ostertagia infections. These included the persistence and increased prominence of lesions appearing first in the acute infection, including hyperplasia of mucous cells, increased mucosal thickness, and mucous metaplasia and de-differentiation (Armour, 1966; Ritchie, 1966; Poynter,1966; Durham and Elliot, 1976; Snider et al, 1988). Prominent periodic nodular hyperplasia may cause the mucosa to assume a scalloped appearance on cross-section, as described in Ost. circumcincta infections of gerbils (Court et al, 1988) and noted in the present study. However, the focal fibrosis and extraglandular larvae reported occasionally by other investigators (Snider et al, 1981; Court et al, 1988) were not seen. The tendency of these hyperplastic and metaplastic changes, together with the augmented inflammatory infiltrate, to chronically displace functional mucosa is an important aspect of long-term Ostertagia infections (Snider et al, 1983). For some gastric trichostrongylids, minimal clinical effects have been observed despite the presence of marked nodular abomasitis associated with numerous mucosal larvae (Pletcher et al, 1984; Al-Qaisy et al, 1987; Al-Zubaidy et al, 1987). However, chronic gastric structural and inflammatory alterations have often been held partly responsible for the suboptimal performance of Ostertagia-infected domestic ruminants (Armour and Ogbourne, 1982; Gibbs and Herd, 1986; Symons, 1989).

The particular pathogenicity of the inhibition-prone strain of Obeliscoides used in the study is indicated by both the persistence of occult or senescent infections and the lesions associated with them. Many features of the lesions observed in this study have been previously associated with either inhibited larvae or with nonpermissive infections. Nodular hyperplasia was prominent within the pyloric zone even when few Obeliscoides were present and although gross lesions and all histological sections of Obeliscoides were from the fundic zone. Similar hypertrophic changes have also been reported in the pyloric zone of gerbils, even after these nonpermissive hosts had eliminated Ost. circumcincta infections (Court et al, 1988). After noting the similarities of lesions induced in ruminants by inhibited Ostertagia spp. to those occurring in nonpermissive hosts, Snider and

coworkers (1983; 1985) proposed the laboratory rabbit as a model for understanding the pathogenesis of mucosal hyperplasia and other chronic lesions.

Distinguishing features of the present Obeliscoides isolate

The lesion characteristics and poor maturational success of the present Obeliscoides isolate indicate that laboratory rabbits were relatively nonpermissive hosts, despite their proven value in the passage of other isolates, suggesting that an enhanced tendency to arrest development was a genetic feature of this particular isolate. That the present isolate differs genetically from those previously studied is a possibility which must be considered. Three factors favor the individuality of this isolate's genetic expression: (1) pathogenicity, (2) geographic location, and (3) isolation from the natural host at a low point in host's cycle.

(1) pathogenicity

Obeliscoides isolates with little tendency to inhibit are more likely to be successfully passaged in laboratory rabbits, especially if they are relatively nonpathogenic to their new host. Such isolates are therefore more likely to be reported in the literature in comparison to pathogenic, inhibition-prone strains such as the present one. A genetically based loss of the ability to arrest development during laboratory rabbit passage has been shown for the University of Guelph (1966) Obeliscoides isolate (Watkins and Fernando, 1984). Percentages of L3 dosages found to inhibit development in the early L4 stage in response to cold exposure dropped from 80% to 15% over 6 years of rabbit passage (1972 to 1978) despite the periodic refrigerator storage of infective larvae for up to 18 months (Watkins, 1982; Watkins and Fernando, 1984). Similarly, percentage arrest dropped from 24% to 5% over 9 years (1961 to 1970) of experimental passage for the Weybridge strain of bovine Ost. ostertagi (Michel et al, 1973). A fresh field isolate of Ost. ostertagi was found to arrest development upon cold exposure to a far greater extent than the laboratory strain (Armour and Ogbourne, 1982). These findings imply that non-arresting trichostrongylid strains may be less pathogenic for their hosts and produce more eggs, and are therefore more amenable to continuous laboratory passage.

(2) geographic location

The geographic origin of Obl. cuniculi isolates previously passaged successfully in laboratory rabbits has either not been given, or has been eastern or central North America. The geographic distribution of Obl. cuniculi in North America is very broad, including 2 subspecies parasitizing several lagomorph and rodent host (Measures and Anderson, 1983a,b). Three other species of Obeliscoides occur in different lagomorph hosts in eastern Asia (Fukumoto, 1986).

Obeliscoides cuniculi multistriatus has been previously identified from hares collected in Fairbanks, Alaska, the source of the present isolate (Measures and Anderson, 1983b). Identification of trichostrongylids in this study was carried only to the generic level, however, because of the considerable size difference between Obeliscoides adults recovered from the snowshoe hares serving as sources for the initial passages and those recovered after laboratory rabbit passages. Both male and female adult Obeliscoides of hare origin were twice the size of those of rabbit origin, which therefore appeared relatively "stunted" although they were within the size ranges previously given for Obl. cuniculi multistriatus from laboratory rabbits (Measures and Anderson, 1983c).

Further studies of Obeliscoides species from Alaskan hares may be needed to clarify the identity of the present isolate. Both the difficulty with which it was passaged in rabbits and its morphologic features are consistent with a degree of taxonomic uniqueness. Adult male Obeliscoides isolated from hares were somewhat longer (10 to 13 mm) than previously reported from either Obl. cuniculi in North America (means of 7.8 to 9.6 mm : Measures and Anderson, 1983a, c, 1984) or Obl. leporis in Asia (8.5 mm : Fukomoto, 1986), but were not as long as Asian Obl. pentalagi (13.5 mm: Fukomoto, 1986). The Fairbanks locale of the present isolate represents the northwestern extreme of the North American range of Obl. cuniculi. This area is within the corridor of the Bering Sea Land Bridge, route of the hypothesized Pleistocene spread of Obeliscoides, within a Lepus host, from Asia into North America (Measures and Anderson, 1983a; Fukomoto, 1986).

The genetic constitution of particular trichostrongylid isolates may be an important factor in understanding features of their biology and the host responses which they elicit. Northern isolates in particular are often distinguished by their responses to cold temperatures, and there is considerable evidence that these responses are genetically based for Obeliscoides as well as for Ostertagia (Watkins, 1982; Watkins and Fernando, 1984; Frank et al, 1986; Helal, Sinski and Bezubik

1987; Frank et al, 1988). Both eggs and infective larvae of northern isolates of Ostertagia remain viable despite exposure to -18 degrees C and repeated freeze/thaw cycles (Jasmer et al, 1986, 1987), perhaps because they possess either distinctive enzyme systems (Helal, Sinski and Bezubik 1987) or the freeze-avoidance and freeze-tolerant mechanisms demonstrated in other genera (Wharton and Allen, 1989). Not only has trichostrongylid infectivity been maintained after as long as 82 weeks at 4 degrees C (Lesage and Mallet, 1987), it has in some cases actually been enhanced by (shorter periods of) cold storage (Mallet and Kerboeuf, 1986; Kerboeuf et al, 1989). Genetic selection induced in Haemonchus contortus populations by cold exposure has also been related to benzimidazole susceptibility, even in the absence of actual anthelmintic exposure (Kerboeuf et al, 1989).

Different tendencies to inhibit in-host larval development upon cold exposure have been demonstrated for Ost. ostertagi isolates from cattle. When ingested as infective larvae after exposure to cool fall pasture temperatures, significantly greater percentages of a northern isolate inhibited their development when compared to a southern isolate. This differential inhibition response persisted despite geographic transplantation of the isolates, and was determined to be based on actual within-species genetic differences (Frank et al, 1986, 1988). Such findings indicate that trichostrongylid isolates from different geographical locales should be compared only with caution. It may be inappropriate to designate certain strains as "cold-adapted" on the basis of their ability to survive as infective larvae in constant 4 degree- Celsius laboratory refrigerators and to subsequently appear as patent infections within a few weeks of inoculation. Laboratory-passaged strains should probably not be equated with inhibition-prone field strains until further information on both geographic variations and the complex causes of larval inhibition are better understood.

(3) Isolation from the natural host during a cyclic low point

The third factor favoring the individuality of this isolate's genetic expression is the timing of its recovery with relation to the cycle of its natural host. It is conceivable that some its unique features, including poor maturation and egg production, increased tendency toward inhibition, and substantial pathogenicity, may be associated with the circulation of distinctive parasite strains favored at different points in the well-known cyclicity of this natural host population.

The abundance of Obl. cuniculi within wild lagomorphs from a wide range of geographic areas has a pronounced seasonality (Dodds and Mackiewicz, 1961; Bookhout et al, 1971; Andrews et

al, 1980; Boggs et al, 1990). However, most field investigators have not used techniques suitable for the recovery of the inhibited larvae which probably form the basis of this fluctuation (Gibbs et al, 1977; Measures and Anderson, 1983b; Keith et al, 1985, 1986). During the high points of their marked population cycles, not only are lagomorphs easily available for study, but Obl. cuniculi also tend to be more abundant (Dodds and Mackiewicz, 1961; Keith et al, 1985). This suggests that isolates for laboratory passage would tend to be recovered at these times, although this information has not been given for any of the previously isolates. The present isolate was obtained in the spring of 1987, at or just beyond a cyclic "low" point for the interior Alaskan snowshoe hare cycle.

In cyclic host populations, parasite strains with lower egg production levels but very long relative lives may be favored (Shaw and Moss, 1989). Indeed, if a degree of immunity is present within a host population parasitized by inhibition-prone trichostrongylids, the more rapidly maturing nematodes may be those most susceptible to immune expulsion (Watkins and Fernando, 1984). A substantially greater percentage of a low or stable hare population may be older, and therefore have acquired some degree of immunity to Obeliscoides infection (Keith et al, 1986), compared to the large percentage of juvenile, immunologically naive hares in rapidly expanding populations. Both the persistence of the present Obeliscoides isolate as inhibited or stunted forms, and its poor fecundity could reflect the kinds of selection pressures seen in the more immune host population.

In immune and nonpermissive hosts, gastric trichostrongylids often fail to persist for more than a few weeks post-inoculation despite the persistence of pathogenic effects (Snider et al, 1981, 1983, 1985; Gibbs and Herd, 1986; Wiggins and Gibbs, 1987, 1990; Court et al, 1988; Seaton et al, 1989). If not eliminated, development is almost inevitably halted just prior to the appearance of fertility rather than at an earlier stage (Zebrowska-Plata, 1980; Snider et al, 1985; Court et al, 1988; Wagland et al, 1989; Conder et al, 1990). Distinctive morphological features that including stunting may also appear (Fox, 1976; Snider et al, 1985; Court et al, 1988; Wagland et al, 1989; Conder et al, 1990). Morphologically distinct nematodes have also been related to the age of either the infective larvae or the in-host stages (Michel et al, 1971; Hong et al, 1987; Lesage and Mallet, 1987).

Among Ostertagia-infected ruminants, egg counts often run a stereotyped course that bears little relation to the actual number of apparently mature trichostrongylids present in the abomasum, particularly if the host has been previously infected (Gibbs and Herd, 1986). The fecundity of apparently mature female Obeliscoides may also be regulated by controls on ovulation (Helal, Sinski

and Bezubik 1987) interacting with host immune factors (Fox, 1976), as well factors related to dose size (Russell et al, 1966; Helal, Sinski and Bezubik 1987) and larval cold exposure (Mallet and Kerboeuf, 1986; Helal, Sinski and Bezubik 1987). The egg production abilities of Obeliscoides which mature from inhibited larvae are reduced (Russell et al, 1966) even when the larvae are transplanted to naive hosts (Fox, 1976), but may continue for as long as one year (Watkins and Fernando, 1984). However, primary infections of a non-inhibiting Obeliscoides strain within 4 years of passage maintained patency for up to 9 months, and secondary infection patencies persisted beyond 6 months (Worley, 1963).

The isolation of persisting nematodes from senescent or occult infections has not previously been reported for Obeliscoides. The tendency of the present isolate to persist indicates that a substantial percentages of inoculated larvae survived for months as inhibited forms, and that their maturation was strictly regulated. Such regulation could be a mechanism for adjusting, prolonging or deferring egg production, and may involve both intrapopulation dynamics of the particular isolate as well as host-parasite interactions.

Conclusions

The characteristics of the present isolate provided a unique opportunity to study the gastric lesions associated with chronic or inhibited trichostrongylid infections. As seen previously in short-term nonpermissive Ostertagia infections (Snider et al, 1985; Court et al, 1988), the mere presence of arrested larvae and stunted adult Obeliscoides over a period of weeks or months was sufficient to generate significant inflammatory and structural changes within the gastric mucosa. Neither continuing larval challenge nor successful maturation and egg production were apparently necessary for this isolate's pathogenicity for laboratory rabbits. As economic models for ruminant Ostertagia, such infections may be valuable in attempting to separate pathogenetic mechanisms associated solely with host's immune responses from those associated with the parasite's biological requirements for maturation and reproduction.

CHAPTER 2

SERUM CONSTITUENT CHANGES IN RABBITS INFECTED WITH THE GASTRIC TRICHOSTRONGYLID OBELISCOIDES

INTRODUCTION

Gastric trichostrongylid nematodes of the genus Ostertagia are among the most common and widespread parasites of domestic cattle and sheep. These parasites cause considerable production losses, and the physiologic alterations they induce in their hosts not only assist in clinical diagnosis, but also provide important clues toward understanding their deleterious effects (Holmes, 1985). Investigations utilizing pair-fed ruminants and laboratory model species have demonstrated that the losses are not simply the result of the inappetence and reduced intake frequently associated with parasitism, but of actual alterations in the metabolism of proteins and certain electrolytes (Sykes, 1987; Symons, 1989). Serial sampling of both the protein and electrolyte constituents of the blood over the course of infections has helped elucidate what these changes are, when they occur, and how they are related to both the immune response of the host as well as to the changing needs of the developing and reproducing parasite.

In Ostertagia spp. infections of cattle and sheep, the primary serum constituent changes are related to the apparent loss of protein, primarily albumin, into the gastrointestinal (GI) lumen (Holmes, 1985; Symons, 1989). When infections are established by a single dose of infective larvae, these changes are most apparent during post-infection week (PIW) 3 (Horak and Clark, 1967), but may not be present if small doses of larvae are given (Ritchie et al, 1966). Most investigations of the effects of trichostrongylids on serum constituents, however, have involved repeated doses of infective larvae, over periods of weeks or months. When Ostertagia spp. are given alone, decreased serum protein, albumin or albumin:globulin (A/G) ratio values have been observed only at the highest doses, between 4 and 12 weeks after infections commenced (Coop et al, 1977; Sykes et al, 1988; Symons et al, 1981). In a study involving a 20-week natural pasture exposure of calves to Ost.

ostertagi, Parkins et al (1982b) found that significant decreases in serum protein and albumin persisted during the following 18-week housed period.

The effects of Ostertagia spp. infections on the electrolyte concentrations of cattle and sheep are less clear than the protein effects. While some investigators have found decreased serum calcium (Ca) and phosphorus (P) concentrations in conjunction with Ostertagia infections (Horak and Clark, 1967; Coop et al, 1977 and 1981; Sykes et al, 1977), the levels have remained unchanged in other studies (Jennings et al, 1966; Coop et al, 1977 and 1981; Sykes et al, 1988). Serum chloride (Cl) and bicarbonate were unaffected by Ost. ostertagi infections in cattle despite diarrhea and electrolyte changes within the gastric lumen (Jennings et al, 1966).

In many investigations of the pathogenesis of trichostrongylids established by natural pasture infection, intestinal genera are present in addition to gastric Ostertagia. Intestinal trichostrongylids, particularly Trichostrongylus colubriformis, have been shown to alter serum constituents in many studies (Sykes et al, 1988; Symons, 1989). The serum of ruminants harboring intestinal trichostrongylid demonstrates a decrease in some serum constituents, including albumin, P and sometimes Ca, and an increase in other substances, including blood (serum) urea nitrogen (BUN) (Reveron et al, 1974; Coop et al, 1976; Steel et al, 1980; Sykes et al, 1988; Symons, 1989). Other serum constituents have rarely been studied, although Brown et al (1989) noted no changes in magnesium (Mg) over an 18-week post-infection period in sheep given both Ost. circumcincta and T. colubriformis, despite demonstrated depressions of serum Ca and P.

The successful laboratory animal models of ruminant trichostrongylid infections have usually involved intestinal rather than gastric genera. T. colubriformis has been intensively studied (Lyons et al, 1987), and Maclean et al (1987) demonstrated a reduction in serum total protein (TP) and albumin in infected gerbils, but few other reports present information on the serum constituent changes occurring in infected lab animals. Ostertagia spp. of cattle or sheep origin has been established in laboratory rabbits Oryctolagus cuniculus, but have failed to mature and reproduce normally (Wood and Hansen, 1960; Zebrowska-Plata, 1980; Snider et al, 1985). Instead, rabbits infected with the related hare trichostrongylid Obeliscoides cuniculi have been proposed as an efficient model system for studying various aspects of ruminant Ostertagia spp. infection (Worley, 1963; Russell et al, 1966; Sollod et al, 1968; Sollod and Allen, 1971; Fernando et al, 1971; Hutchinson et al, 1972; Michel et al, 1975). Although Obl. cuniculi has been successfully passaged in at a number

of laboratories, previous investigations have concentrated on parasitological or immunological features of the infections (Worley, 1963; Russell et al, 1966; Sollod et al, 1968; Stockdale et al, 1970: Sollod and Allen, 1971; Fernando et al, 1971; Hutchinson et al, 1972; Michel et al, 1975; Fox, 1976: Measures and Anderson, 1983c; Watkins and Fernando, 1984; Helal et al, 1987a, b; Wedrychowicz et al, 1988).

The suitability of Obeliscoides-infected rabbits as models for Ostertagia-infected ruminants will obviously depend on the parasite's ability to induce in the rabbit host the same type of physiological effects, within the same general time frame, that Ostertagia spp. induce in ruminant hosts. Serum constituent changes provide one means of analyzing the type and time course of these effects. The goal of the present study was to determine if infections by a recent Alaskan isolate of Obeliscoides sp., obtained from snowshoe hares Lepus americanus and passaged in standard laboratory rabbits Oryctolagus cuniculus (Nielsen, Chap. 1), induced changes in any of 10 serum constituents compared to uninfected rabbits. In addition, the long persistence of the present isolate, up to 45 weeks in infected rabbits, enabled serial serum sampling at a number of points in the course of both primary and secondary (repeat) infections. In a related study, serum constituent changes reported here were contrasted with changes in the concentration of nitrogen, Ca and P in segments of the gastrointestinal (GI) tract and in feces of infected rabbits (Nielsen, Chap 4).

MATERIALS AND METHODS

Animals

Standard random-bred New Zealand White rabbits were acquired as weanlings (6 to 7 weeks of age) from a commercial supplier[6] and were habituated to the colony schedule for at least one month prior to larval inoculation. At inoculation, the 17 rabbits (8 males, 9 females) in this study were between 10 and 54 weeks of age and weighed 1.8 to 4.5 kg; at euthanasia, rabbits ranged from 27 to 85 weeks of age and weighed 3.4 to 5.3 kg. Euthanasia was accomplished by preliminary anaesthesia for intracardiac blood collection using the xylazine-ace/ketamine protocol described below, followed by a post-sampling intracardiac injection of concentrated pentobarbital

[6]R & R Rabbitry; Stanwood, WA 98292

(approximately 150 mg/kg body weight (BW)).

GI parasitism was monitored with regular fecal flotation examinations. The 9 incoming rabbits with patent coccidiosis were treated with a 10- to 14-day course of oral sulfaquinoxaline (SQXN) in drinking water at a dosage of .05 to .15 g/kg BW, and tested repeatedly negative prior to admission to the main colony. Twice during the study, it became necessary to treat the entire colony with oral SQXN after a larval inoculation: 13 of the total 94 blood samples were collected during these treatment periods. None of the 17 rabbits in the present study had gross or histopathological lesions of either intestinal or hepatic coccidiosis at necropsy.

Housing and feeding

Rabbits were held on a 12 hour light/12 hour dark (7 am, 7pm) cycle, individually housed in stainless steel cages, and were fed daily between 0930 and 1030. All rabbits were fed a limited quantity (130-170 grams, depending on age) of commercial pelleted rabbit chow[7] as a daily ration. All rabbits entirely consumed this amount of food within 24 hours (h) when healthy.

Rabbits were considered anorectic if more than one-third of their ration remained in their feed hopper after 24 h (i.e. at the next scheduled feeding). After weighing, the residual chow was discarded, and fresh chow was provided in an amount slightly exceeding that which had been consumed the previous day, until the rabbit's consumption returned to the usual daily level.

Parasites

Infective larvae (L3) of Obeliscoides sp. were initially obtained by culturing colonic contents and feces of three patent snowshoe hares (Lepus americanus) collected in Fairbanks, Alaska, and later by culturing first-, second- and third-passage L3 from the feces of infected colony rabbits as previously described (Nielsen, Chap. 1). Colonic and fecal material was held at room temperature for 12 to 18 days in a moist vermiculite mixture, and viable L3 were extracted in a Baermann apparatus for 24 h. Infective larvae were introduced into new hosts either immediately (storage time of "0 weeks") or after holding for variable periods (up to 28 weeks) in water in a 4-degree C

[7]Complete Rabbit Blend, Purina Mills, Inc.; St. Louis, MO 63166

refrigerator.

Larval inocula were introduced into unanesthetized, restrained rabbits by oral dosing with 1 or 2 gelatin capsules, or into lightly anesthetized (acepromazine 1.0 mg/kg BW; ketamine 25 mg/kg BW) rabbits by a gastric tube (size No. 5 French). Dosages of infective material ranged from 1000 to 50,000 L3 (Appendix A). Number of larvae per infective dose or their passage number were not considered as factors in the present study: L3 used to generate infections came from both original Lepus infections (n=3) and first- and second-passage infections in colony rabbits (Nielsen, Chap. 1). Patency was monitored via fecal flotation, and the presence of adult and immature Obeliscoides were confirmed at necropsy.

Experimental groups

Of the 17 rabbits from which serum samples were drawn for constituent evaluation, 9 rabbits experienced only a single (primary) infection, 4 rabbits were sampled only during their second (repeat) infection, 3 rabbits were sampled during both their first and second infections, and 1 rabbit was never infected. The 94 serum samples obtained at various times from these 17 rabbits were divided for analysis into 3 experimental groups: 16 "control group" sera, 56 "primary-infection group" sera, and 22 "secondary-infection group" sera.

The control group consisted of 16 sera drawn from 6 rabbits either prior to larval or water inoculation (6 sera), or after sham inoculation with water (10 sera). At the time the control sera were collected, rabbits ranged in age from 20 to 29 weeks, weighed 3.3 to 4.1 kg, and were consuming 130 to 145 gm of pelleted feed daily.

The primary-infection group consisted of 56 sera drawn from 12 rabbits after a single exposure to infective Obeliscoides larvae. Rabbits ranged from 10 to 27 weeks of age at primary infection, weighed 1.8 to 4.0 kg, and were consuming 130 to 170 gm of pelleted feed daily in the weeks immediately prior to inoculation. Of the 12 primary infections monitored, 4 were generated by original Lepus isolates, 2 by first-passage L3, 5 by second-passage L3, and one by third-passage L3. Larvae were stored for periods of 0 to 28 weeks, with most inocula made up of larvae which had been accumulated over a period of weeks. Dosages ranged from 1000 to 45,000 L3, and were administered via gelatin capsule (2 cases) or gastric intubation (10 cases).

The secondary-infection group consisted of 22 sera drawn from 7 rabbits after the reintroduction of Obeliscoides larvae to animals which had already been inoculated once. In no case were larvae reintroduced to their original host sources. The interval between the first and second larval inoculations ranged from 33 to 304 days (5 to 44 weeks). Rabbits ranged from 23 to 54 weeks of age at secondary infection, weighed from 3.5 to 4.5 kg, and were consuming 130 to 160 gm of pelleted feed daily in the weeks immediately prior to inoculation. Of the 7 secondary infections monitored, 2 were generated by original Lepus isolates, 2 by first-passage L3, and 3 by second-passage L3. Larvae were stored for periods of 0 to 22 weeks, again with most inocula made up of larvae accumulated over a period of weeks. Dosages ranged rom 2600 to 54,400 L3, and were administered via gelatin capsule (2 cases) or gastric intubation (5 cases).

Duration of sampling

Inoculated rabbits were observed for between 35 and 313 days, i.e. to post-inoculation week (PIW) 45. The single sham-inoculated rabbit was euthanized 5 weeks after water inoculation, when at 29 weeks of age and weighing 4.0 kg.

The 10 primary-infection group rabbits were euthanized between PIW 5 and 41. At that time, rabbits were between 28 and 50 weeks of age and weighed from 3.4 to 4.3 kg. The 7 secondary-infection group rabbits were euthanized between PIW 9 and 45. At that time these rabbits were between 32 and 91 weeks of age and weighed 3.7 to 5.2 kg.

Sample collection

A total of 94 postprandial serum samples from 17 rabbits were evaluated. All samples were collected in the afternoon, between 1.0 and 5.5 hours after food was offered at the regular time. For 16 rabbits, up to 12 sequential blood samples of 1 to 5 ml were obtained from the marginal ear vein or from the central artery of the ear, with xylene (followed by an ethanol wash and water rinse) used to distend the vessels. For all 17 rabbits, blood samples of more than 5 ml were also collected via cardiac puncture while animals were anesthetized (xylazine 5 mg/kg BW, ketamine 60 mg/kg BW, acepromazine 1 mg/kg BW) immediately prior to euthanasia. For one rabbit the pre-necropsy sample was the only one available.

Two additional "control, fasted" samples, not included with the control group sera, were collected from uninfected rabbits which had been briefly fasted. These two samples were collected on a single occasion, when neither of the rabbits to be sampled were fed at the usual time. Instead, at 1.5 and 2.0 hours beyond that time, both were anesthetized with acepromazine (1 mg/kg) and ketamine (25 mg/kg), and blood was collected from the marginal ear vein.

Blood samples were collected into plain glass culture tubes and allowed to clot overnight in the refrigerator. Serum was pipetted from the packed cells and frozen within 24 hours of collection in a low-temperature freezer (minus 60 degrees C), where it was held for periods of up to 3 years.

Serum chemistry evaluation

Ten serum constituents were measured: (1) blood urea nitrogen (BUN); the ionized electrolytes (2) sodium (Na), (3) potassium (K), (4) chloride (Cl), (5) calcium (Ca), and (6) magnesium (Mg); and also (7) inorganic phosphate (IP), (8) total protein (TP), (9) albumin (Alb), and (10) the albumin-to-globulin ratio (A/G). All samples were measured on an Ektachem 700 Analyzer[8], which makes use of specific multilayered test-specific slides containing analytic elements coated on a polyester support (Eastman Kodak Co., 1986, 1987, 1989). Because matrices are designed to accommodate human serum, reference ranges for other species must be determined separately (Lasky et al, 1989), as was done for control sera in the present study.

Using the Ektachem 700, 6 of the 10 constituents were analyzed by end-point colorimetric techniques: BUN (urease-catalyzed hydrolysis, ammonia indicator dye), Ca (Arsenazo III dye in pH 5.6 buffer), Mg (Ca-chelation and formazan dye derivative in pH 9.75 buffer), IP (ammonium phosphomolybdate method at pH 4.2, heteropolymolybdate blue complex), TP (biuret reaction using copper sulfate, tartaric acid, and lithium hydroxide), and Alb (bromcresol green dye-binding)(Eastman Kodak, 1986, 1987). The A/G ratio was calculated using a globulin determined by difference (TP minus Alb). Three of the 10 constituents were measured using the E-700's potentiometric methodology: Na (methyl monensin ion-selective membrane), K (valinomycin ion-selective membrane), and Cl (silver chloride ion-selective electrode)(Eastman Kodak, 1986, 1987, 1989).

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[8]Eastman Kodak Co., Technical Products Div.; Rochester, N.Y. 14650

Of the 940 rabbit serum constituent values measured, only a single value was not contained well within the "dynamic range" performance characteristics (for human serum) of the E-700 Analyzer (Eastman Kodak, 1986, 1987, 1989). A single serum Ca concentration (the highest observed in the study) measured 16.4 mg/dL, while the stated dynamic performance range was 1.0 to 16.0 mg/dL (Eastman Kodak, 1987). Three-fold dilution of the sample, however, yielded an identical value. No other sera required dilution for analysis.

Construction of normal ranges, statistical analysis

Each of the 10 different serum constituents were analyzed separately for each of the 94 serum samples within the study. The 16 "control sera" were collected either prior to larval inoculation (6 sera from 4 rabbits) or after sham inoculation (via gastric intubation) with plain water only (10 sera from 3 rabbits), and were used to calculate control means and deviations after a pooled t-test determined there was no significant ($P < .05$) difference between the two groups for 9 of the constituents. For the tenth, a normal range for serum K was constructed from all 16 of the concentrations observed in uninfected rabbits even though water inoculation appeared to slightly increase serum K concentration. Serum K increased from 6.0 mMol/L (before) to 6.4 mMol/L (after), because a single rabbit with slightly higher values contributed disproportionately to the latter group. All 16 of the observed potassium concentrations were well within the a range of normal means observed by other investigators.

"Control ranges" were constructed to embrace 95% of values found in normal rabbits (mean ± 2 SD) as recommended by Winkel and Statland (1984) after control groups were determined to have Gaussian distributions, since greater precision is obtained by using parametrically estimated reference intervals for clinical chemistry values (Harris and DeMets, 1972; Boyd and Lacher, 1982). Constituents of 56 sera collected during primary infections ("primary infection group") and 22 sera collected during secondary infections ("secondary infection group") were evaluated by PIW relative to the appropriate control group mean using the "Microstat I" statistical package[9] for initial one-way analysis of variance. Differences at the $P < .05$ level were further analyzed by Duncan's multiple

[9]Ecosoft, Inc.; Indianapolis, IN 46220

range test[10], enabling multiple comparisons among sample means (Ott, 1977). When a particular mean for one of the constituents differed significantly ($P < .05$) from the appropriate control group mean, the deviation was considered valid if and only if the mean also fell outside both (1) the control range (2 standard deviations above and below the control mean), and (2) the range actually observed for the 16 control sera (Table 2.1).

The same analytic methods were used to compare the control group (n=16) and the "fasted, control group" (n=2) with an "anorectic group". This last group was a subset of 6 of the 94 postprandial sera defined by a sampling time within one day of clinical anorexia. All six samples were collected during PIW 1, 2, or 3 of primary infections.

RESULTS

Uninfected rabbits

Table 2.1 summarizes the evaluations of 16 (postprandial) control sera for each of the 10 serum constituents, including the observed range, the calculated means and standard deviations, and a "control range" of 2 standard deviations above and below the mean for each constituent. For BUN and TP, the observed range extended slightly below the 2 SD control range, while for the A/G ratio the observed range extended slightly above it.

Table 2.1 also lists average coefficients of variation (CV), which were less than 3% for all constituents except the A/G ratio at less than 5%. CV's were based on 9 sera (37 replicates), from both control and infected rabbits, which were replicated between 3 and 6 times at the beginning, middle and end of the analyses.

When the serum constituent values for the 2 uninfected "fasted, control" rabbits were compared to the postprandial control ranges, there was a tendency for both BUN (mean 15.5 mg/dL) and Ca (mean 11.7 mg/dL) to fall below the range and for IP (mean 6.2 mg/dL) to exceed the range.

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[10]D. Reyna, copyright 1983

TABLE 2.1: CONTROL VALUES FOR 10 SERUM CONSTITUENTS, NEW ZEALAND WHITE RABBITS.

Constituent (units)	{Observed range}	mean#	(SD)	control range (mean +/-2SD)	CV## (aver. %)
1 BUN (mg/dl)	{15-27}	23.2	(3.63)	15.9-30.5	2.70
2 Na+ (mmol/l)	{135-146}	140.2	(3.24)	133.7-146.6	2.93
3 K+ (mmol/l)	{5.6-7.1}	6.26	(0.444)	5.38-7.15	2.63
4 Cl- (mmol/l)	{100-110}	105.5	(2.71)	100.0-110.9	1.29
5 Ca++ (mg/dl)	{13.1-14.6}	13.88	(0.511)	12.85-14.90	1.23
6 Mg++ (mg/dl)	{1.7-2.4}	2.07	(0.912)	1.69-2.45	2.61
7 phos (mg/dl)	{2.4-4.9}	3.39	(0.796)	1.80-4.99	2.32
8 TP (g/dl)	{4.7-5.9}	5.42	(0.308)	4.80-6.04	2.10
9 alb (g/dl)	{3.1-4.3}	3.74	(0.320)	3.10-4.38	2.75
10 A/G (ratio)	{1.9-3.0}	2.26	(0.301)	1.65-2.86	4.95

\# Means, standard deviations (SD) and ranges based on 16 control sera;
Coefficients of variation (CV) based on 9 sera replicated 3 to 6 times.

Moderate hemolysis was examined as a potentially distorting factor for 6 of the 37 replicated sera and 11 of the 94 test sera. Serum K values tended to increase directly with the amount of hemolysis in the replicate, with a CV of 7.2% characterizing the most hemolyzed samples. Serum Ca, TP, Alb and the A/G ratio also tended to increase to a minor extent in more hemolyzed samples. However, none of the 6 replicate and 11 test sera with moderate hemolysis had serum K, Ca, TP, Alb or A/G values which exceeded the control range, and none were found to be a factor in the significant differences reported in infected rabbits.

Infected rabbits

Of the 10 serum constituents evaluated for up to 45 weeks after inoculation with Obeliscoides larvae (Table 2.2), only 3 constituents assumed values at any time which were considered to be significantly beyond the appropriate control range, as previously defined. These were: (1) Mg in both primary and secondary infections, (2) K in primary infections, and (3) Ca in secondary infections.

Serum Mg concentrations significantly ($P < .05$) exceeded the control range in both primary infections, during PIW 8, and in secondary infections, during PIW 11 - 15 (Table 2.2). Serum Mg values were higher than control mean concentrations during 3 additional periods: primary infections during PIW 6, and secondary infections, during PIW 6 and 7. In all 5 periods, average serum Mg values equaled or exceeded 2.5 mg/dl; the highest individual observation, 2.8 mg/dL, was a primary infections during PIW 11.

Average serum K concentration fell significantly below the control range only in primary infections during PIW 5, when it reached 5.2 mg/dl. Mean serum potassium values differed from the control mean during PIW 2 and 11-15 as well, but remained within the control range. There were no significant differences in serum potassium values during secondary infections.

Serum Ca values exceeded the control range only in secondary infections during PIW 1 and 2, including a value of 16.8 mg/dL in one of the 3 individuals sampled. The average serum Ca concentrations observed in primary infections during PIW 10 also was higher than the control mean, but remained within the control range.

TABLE 2.2: SERUM CONSTITUENTS IN CONTROL AND INFECTED RABBITS arranged by post inoculation week (PIW). Data are expressed as mean ±SE.

Group and PIW interval	n	1 BUN (mg/dl)		2 Na (mmol/l)		3 K (mmol/l)	
control	16	23.2	±0.91	140.2	±0.81	6.26	±0.111
primary infections							
PIW 1	5	19.2	±0.97	141.6	±2.71	6.92	±0.285
PIW 2	7	17.7	±0.78	138.9	±0.94	5.77	±0.144
subgroup 1&2	12	18.3	±0.62	140.0	±1.25	6.25	±0.219
PIW 3	8	18.6	±0.91	140.4	±2.18	6.20	±0.217
PIW 4	5	20.4	±1.36	139.6	±2.94	6.26	±0.214
PIW 5	2	25.7	±0.65	141.1	±4.60	*5.27	±0.135
PIW 6	4	18.5	±1.56	139.3	±1.65	6.78	±0.132
PIW 7	5	19.6	±1.40	139.0	±1.41	6.48	±0.153
PIW 8	4	19.4	±1.57	140.6	±0.94	6.50	±0.212
PIW 10	4	19.8	±2.29	143.5	±1.19	6.60	±0.349
PIW 11-15	8	19.9	±1.41	141.2	±0.98	5.94	±0.225
PIW 35	2	20.5	±1.50	146.5	±0.50	6.35	±0.350
PIW 37,41	2	21.8	±0.25	144.0	±1.50	6.03	±0.075
subgroup 37-41	4	21.1	±0.72	145.3	±0.97	6.19	±0.714
total primary	56						
secondary infections							
PIW 1&2	3	22.0	±1.00	147.0	±7.02	6.27	±0.318
PIW 6	3	21.0	±1.00	143.0	±1.53	6.43	±0.088
PIW 7	3	22.3	±0.88	142.0	±1.16	6.60	±0.252
PIW 8	3	21.3	±0.33	140.7	±0.88	6.47	±0.233
PIW 9&10	3	19.4	±3.24	141.8	±2.04	6.09	±0.304
PIW 11-15	3	23.4	±0.30	143.1	±0.95	6.33	±0.176
PIW 39-45	4	24.3	±0.42	142.7	±0.97	6.11	±0.318
total secondary	22						

* Outside the control ranges (mean ±2SD, Table 1) for that constituent

Table 2.2 serum constituents, page 2 of 4

Group and PIW interval	n	4 Cl (mmol/l)		5 Ca (mg/dl)		6 Mg (mg/dl)	
control	16	105.5	±0.68	13.88	±0.128	2.07	±0.048
primary infections							
PIW 1	5	107.6	±1.57	13.30	±0.321	2.12	±0.116
PIW 2	7	104.0	±1.02	13.09	±0.353	2.13	±0.109
subgroup 1&2	12	105.5	±1.00	13.18	±0.237	2.13	±0.076
PIW 3	8	104.5	±1.84	13.54	±0.713	2.14	±0.093
PIW 4	5	104.2	±2.13	13.84	±0.314	2.20	±0.259
PIW 5	2	102.7	±0.97	13.15	±0.250	2.29	±0.040
PIW 6	4	102.8	±1.70	13.63	±0.229	2.50	±0.122
PIW 7	5	102.6	±1.25	13.74	±0.202	2.44	±0.147
PIW 8	4	104.6	±0.75	13.73	±0.165	*2.59	±0.032
PIW 10	4	105.5	±0.65	14.03	±0.253	2.30	±0.071
PIW 11-15	8	103.2	±0.69	13.14	±0.203	2.41	±0.098
PIW 35	2	108.0	±1.00	13.80	±1.500	2.40	0
PIW 37,41	2	105.0	±0.50	13.10	±0.400	2.40	±0.200
subgroup 37-41	4	106.5	±0.98	13.45	±0.386	2.40	±0.082
total primary	56						
secondary infections							
PIW 1&2	3	109.7	±6.67	*15.07	±0.689	2.07	±0.120
PIW 6	3	108.3	±1.20	13.60	±0.252	2.50	±0.173
PIW 7	3	107.0	±0.58	13.40	±0.116	2.50	±0.252
PIW 8	3	105.7	±1.33	13.67	±0.186	2.40	±0.200
PIW 9&10	3	102.2	±4.87	12.98	±0.507	2.31	±0.047
PIW 11-15	3	104.0	±2.38	13.48	±0.182	*2.61	±0.057
PIW 39-45	4	107.6	±0.82	13.42	±0.196	2.10	±0.201
total secondary	22						

* Outside the control ranges (mean ±2SD, Table 1) for that constituent

Table 2.2 serum constituents, page 3 of 4

Group and PIW interval	n	7 phos (mg/dl)		8 TP (g/dl)		9 alb (g/dl)	
control	16	3.39	±0.199	5.42	±0.077	3.74	±0.080
primary infections							
PIW 1	5	4.10	±0.424	5.34	±0.328	3.72	±0.413
PIW 2	7	3.70	±0.332	5.06	±0.149	3.41	±0.213
subgroup 1&2	12	3.87	±0.257	5.18	±0.159	3.54	±0.206
PIW 3	8	3.43	±0.119	5.43	±0.111	3.56	±0.118
PIW 4	5	3.74	±0.093	5.62	±0.183	3.90	±0.150
PIW 5	2	4.46	±0.190	5.33	±0.125	3.44	±0.035
PIW 6	4	4.20	±0.456	5.65	±0.126	3.65	±0.065
PIW 7	5	3.70	±0.358	5.64	±0.068	3.84	±0.121
PIW 8	4	3.96	±0.275	5.71	±0.083	3.93	±0.085
PIW 10	4	4.05	±0.504	5.63	±0.085	3.88	±0.144
PIW 11-15	8	4.15	±0.261	5.25	±0.065	3.43	±0.085
PIW 35	2	3.35	±0.405	5.95	±0.150	4.05	±0.050
PIW 37,41	2	3.40	0	5.33	±0.225	3.58	±0.075
subgroup 37-41	4	3.38	±0.184	5.64	±0.212	3.81	±0.142
total primary	56						
secondary infections							
PIW 1&2	3	3.50	±0.231	5.87	±0.291	4.27	±0.176
PIW 6	3	3.50	±0.265	5.80	±0.058	3.93	±0.088
PIW 7	3	3.67	±0.203	5.73	±0.033	3.93	±0.033
PIW 8	3	2.93	±0.467	5.53	±0.033	3.67	±0.033
PIW 9&10	3	3.48	±0.400	5.41	±0.146	3.64	±0.135
PIW 11-15	3	2.95	±0.186	5.33	±0.220	3.51	±0.143
PIW 39-45	4	2.76	±0.236	5.70	±0.227	3.92	±0.236
total secondary	22						

* Outside the control ranges (mean ±2SD, Table 1) for that constituent

Table 2.2 serum constituents, page 4 of 4

Group and PIW interval	n	10 A/G ratio	
control	16	2.26	±0.075
primary infections			
PIW 1	5	2.34	±0.375
PIW 2	7	2.13	±0.212
subgroup 1&2	12	2.22	±0.191
PIW 3	8	2.01	±0.180
PIW 4	5	2.24	±0.150
PIW 5	2	1.83	±0.175
PIW 6	4	1.93	±0.125
PIW 7	5	2.12	±0.139
PIW 8	4	2.23	±0.144
PIW 10	4	2.28	±0.202
PIW 11-15	8	1.90	±0.083
PIW 35	2	2.15	±0.250
PIW 37,41	2	2.08	±0.125
subgroup 37-41	4	2.11	±0.116
total primary	56		
secondary infections			
PIW 1&2	3	2.73	±0.067
PIW 6	3	2.10	±0.058
PIW 7	3	2.10	±0.058
PIW 8	3	2.03	±0.088
PIW 9&10	3	2.05	±0.104
PIW 11-15	3	1.93	±0.084
PIW 39-45	4	2.20	±0.129
total secondary	22		

* Outside the control ranges (mean ±2SD, Table 1) for that constituent

Anorectic rabbits

Clinical anorexia was observed in 9 of the 16 infected rabbits, but only within the first 3 weeks of the 45-week experimental period. Seven of 10 primary infection rabbits were anorectic between PID 4 and 16 (PIW 1 through 3), while 2 of 6 secondary infection rabbits were anorectic between PID 1 and 6 (PIW 1). Only 6 serum samples (from 4 individuals) collected during anorexia were available for analysis. All 6 were from primary infections.

Mean values for the 10 serum constituents evaluated in these 6 infected, anorectic samples are presented in Table 2.3, where they are contrasted with mean values for the 2 fasted, uninfected rabbits. Both the fasted and the anorectic groups had BUN and Ca values which were lower than the appropriate control means for postprandial sera. Both BUN and Ca were also below the control ranges in the fasted, uninfected group, but only Ca fell below the control range in the anorectic, infected group.

Mean serum IP concentration in fasted, uninfected rabbits was significantly higher than the means of both the control and the anorectic, infected groups, and was also higher than the control range. Mean IP of the anorectic group was close to the control group mean value.

The major serum protein parameters of the 6 anorectic rabbits (Table 2.3) were uniformly different from those of either the control (Table 2.1) or the fasted groups (Table 2.3). Mean values for TP, Alb, and the A/G ratio were all significantly lower than the control means, although only Alb fell below the control range. Both mean Alb and the A/G ratio were significantly below the means observed in the fasted group. In contrast, the major protein parameters of the uninfected rabbits, including both fasted and control rabbits, were very similar.

DISCUSSION

Control values

Although rabbits are among the laboratory species for which blood collection is routine, only a single study (Burns and deLannoy, 1966) discusses the application of microtechnique to the

TABLE 2.3: MEAN VALUES FOR 10 SERUM CONSTITUENTS, FASTED CONTROL RABBITS AND CLINICALLY ANORECTIC INFECTED RABBITS.

	FASTED CONTROLS (n=2)			CLINICALLY ANORECTIC (n=6)		
Constituent (units)	{Observed range}	mean	SD	{Observed range}	mean	SD
1 BUN (mg/dl)	{15-16}	15.5 **	0.71	{15-18}	16.7 *	1.210
2 Na+ (mmol/l)	{133-141}	137.0	5.66	{134-145}	140.5 -	3.728
3 K+ (mmol/l)	{5.40}	5.40	0	{5.4-7.1}	6.02	0.771
4 Cl- (mmol/l)	{104-111}	107.5	4.95	{99-110}	105.0	3.847
5 Ca++ (mg/dl)	{10.9-12.4}	11.65 **	1.061	{12.2-14.0}	12.77 **	0.647
6 Mg++ (mg/dl)	{1.90}	1.90	0	{1.7-2.6}	2.13	0.356
7 phos (mg/dl)	{6.0-6.4}	6.20 ***	0.283	{2.6-4.9}	3.72	0.999
8 TP (g/dl)	{4.9-5.3}	5.10	0.283	{4.5-5.2}	4.83 *	0.281
9 alb (g/dl)	{3.4-3.8}	3.60	0.283	{2.7-3.7}	3.03 **	0.345
10 A/G (ratio)	{2.2-2.6}	2.40	0.283	{1.5-2.4}	1.72 *	0.349

* Significantly lower than control mean (P<.05) but within 2 SD control range
** Significantly lower than control mean (P<.05) and below 2 SD control range
*** Significantly higher than control mean (P<.05) and above 2 SD control range

determination of serum chemistry values in this species. The Ektachem 700 Analyzer used in the present study had advantages including small sample volume and insensitivity to lipemia and hemolysis, but veterinary information included only canine, feline, bovine and equine reference values (Lasky et al, 1989). Mean control values determined for 8 of the 10 serum constituents obtained from uninfected postprandial rabbits in the present study were similar to those previously reported (Table 2.4). Only IP and the A/G ratio were not contained within the ranges of previously published means, with plausible reasons for this discrepancy discussed below. Therefore, despite the new technology and postprandial collection state used to evaluate serum in this study, the control concentrations obtained from uninfected rabbits were felt to be valid comparative references for determining serum constituent changes in Obeliscoides-infected rabbits.

Mean control concentrations of 4 constituents (Na, Ca, Cl, and Alb) were near the middle of the ranges of means published for normal laboratory rabbits, while those of K and BUN, were near the higher ends of the ranges (Table 2.4). Both K and BUN concentrations may have increased slightly during the period of refrigerator storage for clot separation (Ladenson et al, 1974; Rossing and Foster, 1980) and both are also known to increase postprandially (Coles, 1974; Statland and Winkel, 1984). BUN concentrations undergo diurnal fluctuations in the rabbit, increasing during the afternoon (Laird, 1974; Fox, 1989), which is when samples were collected in the present study. Mean control concentrations of two other constituents, Mg and TP, were near the lower ends of the previously observed ranges (Table 2.4). Storage artifacts or postprandial sampling are not known to decrease serum Mg (Statland and Winkel, 1984; Riley and Cornelius, 1989). These relatively low values may reflect strain or dietary differences.

The mean control concentration of TP (5.42 g/dL) was low compared to published means, despite a mean Alb concentration positioned near the middle of its range (Table 2.4). In this study, Alb accounted for an average of 69% of the total serum protein, leaving 31% in the globulin fraction (including alpha, beta, and gamma globulins). Most other investigators have reported lower albumin (53 to 65%) and higher globulin (35 to 48%) percentages for normal rabbits (Allen and Watson, 1958; Benjamin, 1978; Kozma, 1980; Yu et al, 1979; Worstmann, 1961; Fox, 1989). Reported absolute concentrations of serum globulin range from 1.7 to 3.7 g/dL (Hudgins et al, 1956; Worstmann, 1961; Kozma et al, 1974; Laird, 1974; Yu et al, 1979), while in the present study the mean globulin concentration for control sera (found by subtracting mean Alb from mean TP) was 1.68 g/dL. This relatively small globulin fraction accounts for a control mean A/G ratio value (2.3) that is high

TABLE 2.4: PREVIOUSLY REPORTED VALUES FOR 10 SERUM CONSTITUENTS OF NORMAL LABORATORY RABBITS

Constituent (units)	Range of previously reported: means	Individual obs.	References#
1. BUN (mg/dl)	14.3 - 26.7	5 - 31.7	1-10
2. Na+ (mmol/l)	125.4 - 158	100 - 165	1-11
3. K+ (mmol/l)	3.92 - 6.4	3.0 - 9.8	1-11
4. Cl- (mmol/l)	96.5 - 111.8	85 - 120	1-6,8-10
5. Ca++ (mg/dl)	9.50 - 16.8	5.60 - 15.8	1,4-6,8-14
6. Mg++ (mg/dl)	1.87 - 4.03	2.00 - 5.40	2-6,8-12
7. phos (mg/dl)	3.99 - 7.89	2.3 - 8.40	1,2,4-6,9-11
8. TP (g/dl)	4.96 - 7.20	2.87 - 10.13	1-11,15-18
9. alb (g/dl)	2.72 - 4.11	1.37 - 4.1	1-6,8-10,15
10. A/G (ratio)	0.58 - 0.97	0.68 - 1.15	5,8,9

References:

1. Burns and deLannoy, 1966
2. van Kruiningen and Williams, 1972
3. Kozma et al, 1974
4. Laird, 1974
5. Mitruka and Rawnsley, 1977
6. Yu et al, 1979
7. Schuchman, 1980
8. Kraus et al, 1984
9. Kaneko, 1989
10. Loeb and Quimby, 1989
11. Whiting and Quamme, 1984
12. Cheeke, 1987
13. Bourdeau and Hesselton, 1988
14. Goad et al, 1989
15. Hudgins et al, 1956
16. Worstmann, 1961
17. Schalm, 1975
18. Benjamin, 1978

relative to those few previously reported (Table 2.4). A/G values calculated from the data of other investigators range from 0.75 to 1.94 (Hudgins et al, 1956; Allen and Watson, 1958; Worstmann, 1961; Burns and deLannoy, 1966; Van Kruiningen and Williams, 1972; Kozma et al, 1974; Laird, 1974; Benjamin, 1978).

There are three possible explanations for the relatively large A/G value observed for control sera in the present study. (1) Because blood was sampled postprandially the Alb concentration may be higher due to absorptive effects, in comparison with previously published data that is largely from fasted rabbits. In rats, both Alb and TP levels are subject to circadian fluctuations (Nguyen, 1989), and brief fasting markedly decreases albumin synthesis in growing rats (Peters and Peters, 1972). However, longer-term mild dietary restriction has no effect on serum Alb level (Snyder and Towne, 1989), and in the briefly fasted control rabbits in the present study Alb concentration did not decline (Table 2.3). The prolonged digestion of gastric cecoliths in rabbits (Griffiths and Davies, 1963; Cheeke, 1987) could act to buffer intake-related changes in serum levels. (2) Artifactual elevation of serum Alb due to hemolysis or dehydration is a possibility (Riley and Cornelius, 1989), but the single serum sample with moderate hemolysis had an unremarkable Alb concentration,and there was no clinical or serological evidence that control rabbits were dehydrated.

Finally, (3) the unrecognized presence of coccidiosis, one of the most common parasites of laboratory rabbits (Pakes, 1974), may have served to increase the absolute and relative size of the globulin fraction in previously reported studies. Calculations from the data of Allen and Watson (1958) showed that the A/G ratio of coccidia-negative rabbits was more than twice as large as that of infected rabbits even after the latter animals were treated successfully. In the present study, 14 of the 16 control sera were from animals in which coccidial oocysts had never been detected. Because frequent routine fecal examinations were designed to detect helminth ova or oocysts at a level of 0.5 per gram, coccidiosis was immediately recognized and aggressively treated.

Inorganic phosphate (IP) was the second serum constituent examined in this study for which the mean control concentration was below the published range of means, although it remained within the broader range of previous observations (Table 2.4). Besides being affected by differences in rabbit age, strain and ration (Kozma, 1974), IP is a relatively variable serum component subject to diurnal rhythms and other influences (Cotlove et al, 1970; Laird, 1974; Riley and Cornelius, 1989). The lower mean value for the control sera could be the result of postprandial sampling. During their

prolonged (> 6 hours) digestion, cecoliths in the gastric lumen are associated with high levels of phosphate buffer (Griffiths and Davies, 1963). Absorption could explain the higher levels of serum IP, 6.2 mg/dL (Table 2.3) observed in the fasted control rabbits in the present study, since the stomachs of most fasted rabbits contain cecoliths unless coprophagy is prevented (Cheeke, 1987). In humans, serum IP concentration is known to decline after eating (Riley and Cornelius, 1989).

Infected rabbits

The serum of rabbits infected with Obeliscoides showed significant alterations in 3 of the 10 measured serum constituents: K, Mg, and Ca. Alterations in serum protein parameters were significant among infected rabbits with clinical anorexia, discussed below. All these changes in serum concentrations were apparent only in the early weeks of infections, prior to PIW 15, and were temporally associated with both severe gastric lesions and with the parasites' maturation and early patent periods (Nielsen, Chap. 1). No serum constituent changes were associated with the persisting populations of immature, stunted, or infertile Obeliscoides, and with the chronic gastric lesions they induced (Nielsen, Chap. 1), during PIW 15 to 45.

Significant alterations in serum concentrations of both K and Mg were observed in infected rabbits during PIW 5 to 15. Among rabbits with primary infections, significant increases in serum Mg concentration were found during PIW 6 and 8, and significant decreases in serum K were found during PIW 5 (Table 2.2). Coincidental elevations of the polypeptide hormone gastrin also occurred during PIW 6 and 7 in these rabbits (reported separately: Nielsen, Chap. 3). Since the gastrin increase may indicate regional changes in pH within the anterior GI tract occurred in this time (Nielsen, Chap. 4), the normal ionic framework for the secretion and absorption of both K and Mg may have been affected.

Mg has complex and incompletely understood relationships with P and particularly with Ca, and Mg concentrations in mammalian serum tend to increase when Ca levels fall due to renal antagonism between these electrolytes (Capen and Rosol,1989). In a study of lab rabbits (Whiting and Quamme, 1984), the feeding of a high-calcium diet was not accompanied by hypercalcemia, but a 16% decline in plasma Mg was observed. Despite marked alterations in urinary Ca clearance in that study, Mg clearance was unchanged (Whiting and Quamme, 1984). In the present study hypocalcemia did not accompany the observed hypermagnesemia.

In most monogastrics, Mg is absorbed primarily in the cranial jejunum (Strombeck, 1979). Its serum concentrations may be increased if its GI absorption is enhanced, which is facilitated by a reduction in its ionization (Riley and Cornelius, 1989). Increased alkalinity within the anterior GI tract, which is a common feature of ruminant Ostertagia spp. infections (Symons, 1989), reduces Mg ionization and could explain the increased serum concentrations observed in Obeliscoides-infected rabbits in the present study. In ruminants, considerable Mg absorption occurs proximal to the small intestine (Martens and Rayssiguier, 1990) and is greatly enhanced by the addition of certain soluble carbohydrates to the rumen (Giduck and Fontenot, 1984; Thompson et al, 1984). It is also possible that the moderate hypermagnesemia observed here may have been related to altered cecotrophic digestion within the stomachs of infected rabbits. None of the sera with increased Mg concentrations were collected during periods of SQXN treatment, when total mean retention time for liquids was altered by antibiotic exposure (Nielsen, Chap. 4).

During the interval between PIW 6 and 15, serum Mg concentrations increased significantly with respect to the relatively low control value used in this study (Table 2.1), but they remained well within the published range of means for serum Mg in normal rabbits (Table 2.4). There was no discernible relationship between hemolysis or possible storage artifacts and the increased values.

Serum K concentrations were significantly reduced among rabbits with primary Obeliscoides infections during PIW 5, and were also low during PIW 2 and 11-12 although not significantly below the control range (Table 2.2). Increased loss into the GI lumen is one of the primary causes of hypokalemia in most species, even when unaccompanied by other electrolyte abnormalities (Strombeck, 1979; Carlson, 1989; Riley and Cornelius, 1989). Marginally low serum K in PIW 2 coincided with clinical signs of anorexia and failure to reingest cecoliths in some rabbits, but the lowest serum values, during PIW 5, occurred in clinically normal rabbits. The decreased serum K concentrations of the present study remained within the broad range of previously observed normal serum means (Table 2.4). None of the hypokalemic sera were collected during SQXN treatment, which could have increased renal K excretion (Narins et al, 1972).

While hypokalemia in the absence of diarrhea or reduced intake has not been described previously in gastric trichostrongylid infections, increased mucus production is a possible mechanism for this serum alteration. GI mucus is rich in K (10 to 110 mEq/L in man), so that mucoid diarrhea is a common cause of hypokalemia in laboratory species (Riley and Cornelius, 1989). Van Kruiningen

and Williams (1972) observed hypokalemia in association with mucoid enteritis in rabbits. Symons (1989) has discussed increased mucin production as one cause of the endogenous protein loss observed in GI parasitism. Gastric mucous cell hyperplasia is a common lesion in Ostertagia spp. infections of sheep (Armour et al, 1966), cattle (Ritchie et al, 1966), and rabbits (Snider et al, 1985), as well as in Obeliscoides infections (Russell et al, 1966, 1970; Sollod et al, 1968; Bookhout, 1971) In the present study, mucous cell hyperplasia was a prominent gastric lesion in rabbits necropsied prior to PIW 15, and the hypokalemia observed during PIW 5 was temporally associated with Obeliscoides maturation (Nielsen, Chap. 1). The stomachs of rabbits necropsied prior to PIW 15 contained numerous maturing Obeliscoides lying in a thick layer of clear mucus which adhered firmly to the mucosal surface (Nielsen, Chap. 1).

Serum Ca concentrations were significantly increased only during PIW 1 and 2 of secondary infections, and remained within the very broad range of published means (Table 2.4). The rabbit is unusual among mammals in allowing serum Ca concentrations to reach high levels if intake is high, with the excess being accommodated by increased renal fractional excretion (Cheeke, 1987; Goad et al, 1989). Clinical anorexia in some of the secondary infections in the present study did coincide with this period, and the observed hypercalcemia may have been related to decreased water intake and urine production. Although hypercalcemia facilitates gastrin release in most species (Yau, 1982), serum gastrin concentrations remained within the low normal range for the affected rabbits (Nielsen, Chap. 3), suggesting either that the observed hypercalcemia was transient and related to intake/absorption, or that gastrin release in rabbits is relatively refractory to hypercalcemia. Calcium concentrations within segments of the GI tract fluctuated substantially over the course of these Obeliscoides infections (Nielsen, Chap. 4).

While hypocalcemia and hypophosphatemia have been associated with a few high-dose Ostertagia infections, serum Ca and IP levels remained unchanged in both primary and secondary Obeliscoides infections, with the exception of the single hypercalcemic period described above. Most single-dose and low-dose Ostertagia spp. infections in cattle and sheep have also failed to affect serum Ca and IP levels (Jennings et al, 1966; Coop et al, 1977 and 1981; Sykes et al, 1988). Although neither increased Mg nor decreased K have been observed in the serum of Ostertagia-infected ruminants, relatively few studies (Brown et al, 1989) have included an evaluation of these electrolytes.

Anorectic infected rabbits

Clinical anorexia was observed in 9 of the 16 infected rabbits, although sera collected during the anorectic period were available from only 4 individuals. Sera from anorectic rabbits shared significantly decreased serum BUN and Ca concentrations with fasted uninfected rabbits (Table 2.3), indicating that reduced intake alone could account for reduction in both these constituents. In contrast, significantly increased IP was seen only fasted uninfected rabbits, while the anorectic rabbits retained values similar to those of uninfected postprandial controls. As previously noted, both BUN and IP are variable serum components which fluctuate with feeding or fasting (Cotlove, 1970; Coles, 1974; Laird, 1974; Statland and Winkel, 1984; Fox, 1989; Riley and Cornelius, 1989). Decreased serum IP in anorectic rabbits may indicate that a reduction in cecotrophy accompanied the infection-related reduced intake, since cecoliths are rich in P (Nielsen, Chap. 4) and gastric cecoliths are associated with high levels of phosphate buffer (Griffiths and Davies, 1963). Since serum Ca is closely related to intestinal absorption in rabbits (Cheeke, 1987; Goad et al, 1989), it is logical that it would also decrease during fasting, as was demonstrated and discussed by Bourdeau and Hesselton (1988) for cottontail rabbits (presumably Sylvilagus sp.). The observed values for BUN, Ca and IP in all 3 rabbit groups remained well within the ranges of published means (Table 2.4).

Serum protein parameters were significantly different in anorectic Obeliscoides-infected rabbits compared to uninfected rabbits which were either fed (controls) or fasted. Gastric trichostrongylid infections that resulted in clinical anorexia also caused reductions in serum constituents related directly to protein metabolism, including TP, Alb, and the A/G ratio (Table 2.3). TP concentration declined sharply, to 4.83 mg/dL, which was below the 2 SD control range (Table 2.1) as well as the range of previously reported means (4.94 to 7.20 mg/dL) (Table 2.4). Alb concentration was also reduced, averaging 3.03 mg/dL compared to the control value of 3.74 mg/dL (Table 2.1), and, because the mean A/G ratio also declined (from a control value of 2.3 to an average of 1.7), there must have been a substantial increase in the globulin fraction. Similar globulin increases have been encountered with other GI tract lesions in rabbits (Leland et al, 1955; Allen and Watson, 1958; van Kruiningen and Williams, 1972). However, the significant decline in TP concentration indicates that expansion of the serum globulin fraction was not enough to compensate for albumin fraction losses.

Coincident with this apparent increase in albumin loss, fecal N excretion was significantly

increased in all Obeliscoides-infected rabbits during PIW 1 and 2, with this loss continuing to PIW 5 in primary infections (Nielsen, Chap 4). Since serum protein alterations occurred only in anorectic rabbits, many infected rabbits were probably able to compensate for these deleterious parasitic effects. When serum concentrations for all infected rabbits, including both anorectic and nonanorectic, were analyzed together, significant differences in serum total protein, albumin, and the A/G ratio were not apparent. Even among anorectic rabbits, serum protein-related alterations occurred only during the initial phases of the enhanced fecal loss, which is consistent with separate evidence for increased intestinal absorption of protein (N) during PIW 5 to 15 in rabbits with primary infections (Nielsen, Chap. 4).

Decreased serum albumin or A/G ratios and increased serum globulins have frequently been reported to accompany Ostertagia spp. infections in ruminants (Coop et al, 1977; Parkins et al, 1982b; Sykes et al, 1987; Symons, 1989). Steel and Symons (1982) have discussed how increased GI loss of albumin in many trichostrongylid infections is actually accompanied by an increased hepatic synthesis that, at certain stages of the infection, becomes unable to compensate for the accelerated loss. In the present study, the amount of albumin lost did not appear to be large, because the mean concentration remained both within the 2 SD control range for this study and within the range of previously reported means (Table 4). While the kinetics of the albumin pool have been investigated in laboratory animal models species for intestinal trichostrongylids (Symons et al, 1974; Symons, 1989), they have not previously been studied for the gastric genera.

In the present study, serum protein-related constituents and Ca were altered within the first 3 weeks of Obeliscoides infection and were related to anorexia, while changes in serum K, Mg, and gastrin concentrations occurred during PIW 5 to 15 and were not associated with clinical signs although they coincided with severe mucosal lesions (Nielsen, Chap. 1). The sequential timing of these serum constituent changes may be related to biological activities of the gastric nematode population, stage-specific antigens, and the immunological phenomena that accompany them. In particular, the interval between PIW 5 and 15 coincides closely with increases in serum and mucosal antibody titers against surface antigens of Obl. cuniculi larvae and adults that occurs during PIW 5 through 12 (Wedrychowicz et al, 1988).

Conclusions

Obeliscoides-infected laboratory rabbits represent a potential model for the further investigation of serum constituent changes that accompany gastric trichostrongylid infections in commercial ruminants. Endogenous protein losses are a principle cause of production losses in Ostertagia-infected cattle and sheep, and serum constituent changes consistent with this lesion were encountered in the model system. While hypocalcemia and hypophosphatemia were not observed, alterations in serum Mg and K concentrations observed in infected rabbits may be related to enhanced mucus production and ionic changes with the GI lumen that have been difficult to recognize in infected ruminants. Finally, the temporal pattern of these serum alterations in rabbits may help illuminate the pathogenic mechanisms associated with particular phases in the life cycle of gastric trichostrongylids.

CHAPTER 3

SERUM GASTRIN CONCENTRATIONS IN RABBITS INFECTED WITH THE GASTRIC TRICHOSTRONGYLID OBELISCOIDES

INTRODUCTION

In domestic herbivores, gastric parasitism by trichostrongylid nematodes is associated with a wide variety of physiological effects both within the digestive tract and systemically (Fox et al, 1987; Sykes, 1987; Symons, 1989). It is increasingly recognized that Ostertagia species, common parasites of the ruminant abomasum, may exert deleterious physiological effects that reduce productivity even in the absence of clinical disease. It would be more economical to investigate these complex effects in small laboratory model species, but attempts to passage ruminant-origin Ostertagia spp. in rodents and lagomorphs have been relatively unsuccessful (Wood and Hanson, 1960; Zebrowska-Plata, 1980; Snider et al, 1985; Court et al, 1988; Okamoto et al, 1988). Several isolates of the hare trichostrongylid Obeliscoides cuniculi has been repeatedly passaged in the laboratory rabbit (Worley, 1963; Russell et al, 1966; Sollod et al, 1968; Sollod and Allen, 1971; Fernando et al, 1971; Michel et al, 1975; Fox, 1976; Pace and Frandsen, 1982; Measures and Anderson, 1983c; Watkins and Fernando, 1984; Helal, Sinski and Bezubik, 1987; Wedrychowicz et al, 1988). Because this genus is closely related to Ostertagia, Obeliscoides-infected rabbits have been proposed as an economical model system for understanding the pathophysiology of ruminant gastric trichostongylid infections (Worley, 1963; Russell et al, 1966; Sollod et al, 1968; Sollod and Allen, 1971).

In Ostertagia-infected ruminants, deleterious physiological effects may occur prior to or after the time when patency is detectable by standard fecal flotation examinations (Holmes, 1985; Gibbs and Herd, 1986; Ploeger, Van Straalen et al, 1990; Ploeger, Kloosterman, Borgsteede and Eysker, 1990; Ploeger, Borgsteede et al, 1990), and other means of diagnosing occult but costly infections have been tried. Because serum levels of pepsinogen and gastrin may dramatically increase in response to gastric trichostrongylids, evaluation of these levels are diagnostic alternatives. Serum

pepsinogen assays are frequently employed for ruminants in Europe and Australia (Kerboeuf et al, 1981; Schillhorn van Veen, 1988; Hilderson et al, 1989; Ploeger, Van Straalen et al, 1990; Ploeger, Kloosterman, Borgsteede and Eysker, 1990; Ploeger, Borgsteede et al, 1990), but are less available in the United States. Standard determinations of pepsinogen concentration also requires several milliliters of fresh serum (Harvey-White and Allen, 1982), so are less suitable for small animals. Because serum gastrin assays utilize radioimmunoassay (RIA) techniques, they require less than a milliliter of serum, and are useful in laboratory model species.

There are a two major advantages to understanding variations in serum gastrin during trichostrongylid infections. First, the hormone is the only one known to stimulate HCl secretion (Walsh and Grossman, 1975), it exerts a variety of other physiological effects (Yau, 1982), and its increase could account for some of the clinical and subclinical effects trichostrongylids are known to produce in their hosts (Fox et al, 1987). Second, serum gastrin levels have considerable potential as research tools, since they are suitable for laboratory animals, and as diagnostic tools, since commercial RIA kits are available. Although these kits are designed to detect human gastrin, their use is generally applicable to other species because variation in molecular structure shown by this polypeptide is minimal over a broad range of vertebrates (Rehfeld, 1979; Reeve et al, 1981). Commercial human RIA kits have been validated for use in the dog (Gabbert et al, 1984), horse (Young and Smyth, 1983), and pig (Yang et al, 1990), and have also been used to measure gastrin concentrations in cattle (McKellar et al, 1987; Fox, Gerrelli, Pitt, Jacobs, Gill and Simmonds, 1989) and sheep (Blanchard and Wescott, 1985).

RIA techniques have been used to show increases in gastrin concentration during ruminant Ostertagia spp. infections (Anderson et al, 1976, 1981, 1985; Blanchard and Wescott, 1985; Entrocasso, 1986; Fox et al 1987, 1988; Fox, Gerrelli, Pitt, Jacobs, Gill and Simmonds, 1989; Pitt et al, 1988; Reynolds et al, 1979). These increases are associated with an increase in gastric pH which occurs during certain stages of the parasite's life cycle (Titchen, 1982; Anderson et al, 1985; Symons, 1989). However, there is considerable evidence that mature trichostrongylids in contact with the gastric mucosal surface can also increase serum gastrin by an unknown but direct mechanism independent of gastric pH change (Eiler et al, 1981; Anderson et al, 1981; Titchen, 1982; McKellar et al, 1987). An elevated serum gastrin level has been considered as a possible diagnostic indicator not only of type I (direct) ostertagiasis, but also of the type II form, which results from the maturation of inhibited larvae after a period of "arrest" within the deep mucosa (Entrocasso et al, 1986).

Snider et al (1985) discussed the potential role of pH changes and serum gastrin relative to hyperplastic mucosal changes in laboratory rabbits infected with immature Ost. ostertagi of bovine origin, and proposed the rabbit as a model for pre-type II ostertagiasis. In their later studies relating pathological changes to hypergastrinemia, however, cattle were used (Snider et al, 1988). Other investigators studying Obl. cuniculi in laboratory rabbits have not evaluated changes in serum gastrin levels.

The common heptadecapeptide form of gastrin (G-17) from rabbits has been isolated and characterized (Jiang et al, 1988), and differs by a single amino acid from human G-17. Nevertheless, there is only a single report in the literature of the normal gastrin level for rabbits (Chey et al, 1975), which is given as a broad range (100 - 300 pg/ml) with no accompanying information on whether the serum was collected from fasting or postprandial rabbits, age, sex, or number sampled.

To further investigate the utility of Obeliscoides-infected rabbits as a laboratory model system, serum gastrin level evaluation was part of a larger pathophysiological investigation. A commercially available RIA kit (for determining human serum gastrin) was modified to evaluate rabbit serum gastrin. Because fasting was to be avoided as an unnecessary stressor in infected rabbits, the normal serum gastrin level (mean and range) were determined for uninfected postprandial rabbits. This level was then compared with those of rabbits with primary and secondary (repeat) Obeliscoides infections to determine if statistically significant increases, above that induced by feeding, could be detected. The Obeliscoides strain used to generate these infections was recently isolated from Alaskan snowshoe hares, and was characterized by a prolonged, often occult course (Nielsen, Chap. 1). Serial collections of serum were used to evaluate potential chronic effects on gastrin concentration.

MATERIALS AND METHODS

Animals

Standard random-bred New Zealand White rabbits were acquired as weanlings (6 to 7 weeks

of age) from a commercial supplier[11] and were habituated to the colony schedule for at least one month prior to larval inoculation. At inoculation, the 18 rabbits (8 males, 10 females) in this study were between 10 and 54 weeks of age and weighed 1.8 to 4.5 kg; at euthanasia, rabbits ranged from 22 to 91 weeks of age and weighed 3.4 to 5.3 kg. Euthanasia was accomplished by preliminary anaesthesia for intracardiac blood collection using the xylazine-ace/ketamine protocol as described below, followed by a post-sampling intracardiac injection of concentrated pentobarbital (approximately 150 mg/kg body weight (BW)).

Gastrointestinal (GI) parasitism was monitored with regular fecal flotation examinations. Incoming rabbits with patent coccidiosis were treated with a 10- to 14-day course of oral sulfaquinoxaline in drinking water at a dosage of .05 to .15 g/kg BW, and tested repeatedly negative prior to admission to the main colony. Twice during the study, it became necessary to treat the entire colony with oral sulfaquinoxaline after a larval inoculation: 13 (of a total of 98) samples were collected during these treatment periods. Pulmonary lesions consistent with acute bacterial (Pasturella sp.) pneumonia were found in a single study rabbit, which was euthanized 12 day post-infection because of acute dyspnea.

Housing and feeding

Rabbits were held on a 12 hour light/12 hour dark (7 am, 7pm) cycle, individually housed in stainless steel cages, and were fed daily at between 0930 to 1030. All rabbits were fed a limited quantity (130-170 grams, depending on age) of commercial pelleted rabbit chow[12]. All rabbits entirely consumed this amount of food within 24 hours when healthy.

Rabbits were considered anorectic if more than one-third of their usual (pre-inoculation) ration remained in their feed hopper after 24 hours (i.e. at the next scheduled feeding). The residual chow was weighed, and fresh chow was provided in an amount slightly exceeding that which had been consumed the previous day. During anorectic periods, rabbits which drank less than 150 ml daily and appeared to be dehydrated were supplied with SC lactated Ringer's solution at 5 - 15 ml/kg SID or

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[11]R & R Rabbitry, Stanwood, Wash. 98292

[12]"Complete Rabbit Blend"; Purina Mills, Inc., St. Louis, MO 63166

BID as required.

Parasites

Infective larvae (L3) of Obeliscoides sp. were obtained by culturing colonic contents and feces of three patent snowshoe hares (Lepus americanus) collected in Fairbanks, Alaska, and by culturing first-, second-, and third-passage L3 from the feces of infected colony rabbits as previously described (Nielsen, Chap. 1). Colonic and fecal material was held at room temperature for 12 to 18 days in a moist vermiculite mixture, and viable L3 were extracted in a Baermann apparatus for 24 hours. Infective larvae were introduced into new hosts either immediately (storage time of "0 weeks") or after holding for variable periods (up to 28 weeks) in water in a 4-degree C refrigerator.

Larval inocula were introduced into unanesthetized, restrained rabbits by oral dosing with 1 or 2 gelatin capsules, or into lightly anaesthetized (acepromazine 1.0 mg/kg BW; ketamine 25 mg/kg BW) rabbits by a gastric tube (size No. 5 French). Dosages of infective material ranged from 1000 to 50,000 L3 (Appendix A). Number of larvae per infective dose or their passage number were not considered as factors in the present study: L3 used to generate infections came from both original Lepus infections (n=3) and first- and second-passage infections in colony rabbits (Nielsen, Chap. 1). Patency was monitored via fecal flotation, and presence of adult and immature Obeliscoides were confirmed at necropsy.

Samples

A total of 98 serum samples from 18 rabbits were evaluated. All samples were collected in the afternoon, between 1.5 and 5.5 hours after food was offered at the regular time. Up to 11 sequential blood samples of 1 to 5 ml were obtained from the marginal ear vein or from the central artery of the ear for 16 rabbits, with xylene (followed by an ethanol wash) used to distend the vessels.

For all 18 rabbits, blood samples of more than 5 ml were also collected via cardiac puncture while animals were anaesthetized (xylazine 5 mg/kg BW, ketamine 60 mg/kg BW, acepromazine 1 mg/kg BW) immediately prior to euthanasia. For 2 rabbits the pre-necropsy sample was the only one available. Serum samples from rabbits which had been fasted (i.e. not fed at their usual time on the day of euthanasia) were not included in this study.

Blood was allowed to clot overnight in the refrigerator, and was pipetted from the packed cells and frozen within 24 hours of collection. Serum was stored in a low-temperature freezer (minus 60 degrees C) for periods of up to 3 years.

Experimental groups

Of the 18 rabbits from which serum samples were drawn for gastrin evaluation, 10 rabbits experienced only a single (primary) infection, 4 rabbits were sampled only during their second (repeat) infection, 3 rabbits were sampled during both their first and second infections, and 1 rabbit was never infected. The 98 serum samples obtained at various points from these 18 rabbits were divided for analysis into 3 experimental groups: 15 samples were "control group" values, 60 samples were "primary-infection group" values, and 23 samples were "secondary-infection group" values.

The control group consisted of samples from 6 rabbits, including 9 samples taken prior to L3 inoculation and 6 samples taken after sham inoculation (via gastric intubation) with plain water. A pooled t-test failed to show a significant difference in serum gastrin levels between pre-larval inoculation and sham-inoculation samples.

The primary-infection group consisted of 60 samples drawn from 13 rabbits after a single exposure to infective Obeliscoides larvae. Rabbits ranged from 10 to 27 weeks of age at primary infection, weighed 1.8 to 4.0 kg, and were consuming 130 to 170 gm of pelleted feed daily in the weeks immediately prior to inoculation. Of the 13 primary infections monitored, 4 were generated by original Lepus isolates, 3 by first-passage L3, 5 by second-passage L3, and one by third-passage L3. Larvae were stored for periods of 0 to 28 weeks, with most inocula made up of larvae which had been accumulated over a period of weeks. Dosages ranged from 1000 to 45,000 L3, and were administered via gelatin capsule (2 cases) or gastric intubation (11 cases), as described elsewhere (Nielsen, Chap.1).

The secondary-infection group consisted of 23 samples drawn from 7 rabbits after the reintroduction of larvae to rabbits which had already been inoculated once. In no case were larvae reintroduced to their original host sources. The interval between the first and second larval inoculations ranged from 33 to 304 days (5 to 44 weeks). Rabbits ranged from 23 to 54 weeks of age at secondary infection, weighed from 3.5 to 4.5 kg, and were consuming 130 to 160 gm of pelleted

feed daily in the weeks immediately prior to inoculation. Of the 7 secondary infections monitored, 2 were generated by original Lepus isolates, 2 by first-passage L3, and 3 by second-passage L3. Larvae were stored for periods of 0 to 22 weeks, again with most inocula made up of larvae accumulated over a period of weeks. Dosages ranged rom 2600 to 54,400 L3, and were administered via gelatin capsule (2 cases) or gastric intubation (5 cases) (Nielsen, chap.1).

Duration of sampling

Infected rabbits were observed for between 35 and 313 days, i.e. post-inoculation weeks (PIW) 5 to 45, with the exception of the acutely dyspneic animal described below. The single sham-inoculated rabbit was euthanized 5 weeks after water inoculation, when at 29 weeks of age and weighing 4.0 kg.

Nine of the primary-infection group rabbits were euthanized between PIW 5 to 41. At that time, rabbits were between 28 and 50 weeks of age and weighed from 3.4 to 4.3 kg. The tenth rabbit in this group, euthanized after 4 days of anorexia when it became acutely dyspneic due to Pasturella pneumonia, was 12 weeks old and weighed 1.4 kg on post-inoculation day (PID) 12 of a primary infection.

The 7 rabbits from which secondary-infection samples were drawn were euthanized between PIW 9 and 45. At that time rabbits were between 32 and 91 weeks of age and weighed 3.7 to 5.2 kg.

Gastrin radioimmunoassay

Serum gastrin values were determined by radioimmunoassay using rabbit anti-gastrin antiserum, 125-I labeled human synthetic gastrin-17 (G-17), synthetic human gastrin standards, and a human control (synthetic G-17 in human serum, lyophilized) supplied as part of a commercial kit[13]. This kit was specifically selected because it was designed to assay several forms (G-17, G-13, G-34) of human gastrin (Rehfeld et al, 1983), a feature recommended when evaluating ostertagiasis (Anderson et al, 1981). Its anti-gastrin antibody product has been independently verified for minimal

[13]Ventrex Labs Inc., Portland, Maine, 04104; known before August, 1989 as Cambridge Medical Technology Corp., Billerica, Mass., 01865

cross-reactivity with related peptide hormones (Wolfe et al, 1985).

For all 12 assays, the kit-supplied anti-gastrin antiserum was diluted with borate buffer (pH 7.6) to a 70% concentration, which yielded approximately 50% binding in the maximal binding (B-o) tube. The final volume of the incubation mixture was 400 microliters (mcl), including 200 mcl of undiluted test serum; incubation was for 46 to 48 hours at 4 degrees C, to allow complete equilibrium (Walsh, 1974; Hansky and Soveny, 1977; Jaffee and Walsh, 1979; McGuigan and Wolfe, 1982).

After incubation, separation of bound and free radiolabeled antigen was accomplished by precipitation of free 125-I gastrin with dextran-coated charcoal solution, because preliminary trials indicated that the gamma globulin component of normal and test rabbit sera interfered with immune complex precipitation by the kit-supplied goat-anti-rabbit second antibody. Dextran-coated charcoal solution in gelatin buffer was made up biweekly similar to procedures previously described (Hebert et al, 1965; Abraham and Manlimos, 1977; Hansky and Soveny, 1977). One gram of dextran T-70[14] was dissolved in 10 ml distilled water and added to 390 ml of PBS-G (phosphate-buffered saline-gelatin). Ten grams of Norit-A charcoal[15] were added to the mixture by vigorous stirring for about 20 minutes. The resulting suspension was chilled at 4 degrees C for at least 24 hours, and stirred for about 10 minutes immediately prior to use.

For the separation procedure, 1 ml of the dextran-charcoal solution was added to the reaction tube, and incubated at least 5 but less than 10 minutes, then immediately centrifuged at 3500 rpm (4 degrees C) for 15 minutes. The supernate was poured into a separate test tube, and both supernate (with bound labeled complexes) and precipitate (charcoal pellet containing free 125-I gastrin) tubes were counted for from 3 to 7 minutes on a gamma spectrophotometer[16]. Concentration of 125-I-labeled antigen averaged 14,720 cpm per tube; maximal cumulative counts totaled at least 45,000.

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[14]Pharmacia LKB Biotechnology Inc.; Pleasant Hill, CA 94523

[15]Eastman Kodak Co.; Rochester, NY 14650

[16]Micromedic 4/200 plus Automatic Gamma Counter, model 28058, ICN Micromedic Systems: ICN Biomedical Inc., Costa Mesa, CA 92626

The kit-supplied gastrin standards (all Ventrex lot # 12779), at concentrations of 20, 40, 90, 170, 340, and 800 pg/ml, were run separately for each of the 12 assays. These six points were plotted as percentages of the average B-o (replicated at least 3 times) for that assay on semi-log paper and a standard curve (drawn free-hand) was generated. (As an example, the standard curve for Assay #6 is supplied as Figure 3.1). Similarly, the percentage of the bound, labeled antigen present in each test sample relative to the average B-o for that assay was calculated, and the corresponding gastrin concentrations were then determined by reading directly from the assay curve. All standards and test sera were run in duplicate.

Interassay coefficients of variation for the kit-supplied lyophilized "human control serum" (all Ventrex lot #20829) were 4.9%, and for "normal rabbit serum" (aliquots of a purchased bulk frozen "Sterile, Filtered Rabbit Serum"[17]) were 5.2%. Intra- and interassay coefficients of variation for replicates of test samples averaged 7.5% and 5.5% respectively. Serial dilutions of 2 separate high-gastrin test samples were found to parallel the standard curve in the range 35 to 400 pg/ml.

Nonspecific binding values (125-I gastrin plus borate buffer), subtracted from the raw count information for each assay, averaged 362 cpm; rabbit nonspecific binding values (200 mcl normal rabbit serum, 100 mcl borate buffer, 100 mcl 125-I gastrin) averaged 187 cpm (above NSB) for 12 assays. The detection limit, or minimum concentration of added antigen which could be detected in test serum under the conditions and dilutions of this assay at $P < .05$ (by t-test), was 5 pg/ml (86.4% of B-o).

Statistical evaluation

The 98 serum gastrin (pg/ml) values were evaluated by experimental group and by weeks post-inoculation within the primary and secondary groups. Differences in serum gastrin concentrations were considered significant if they deviated from the mean control (uninfected) value at $P<.05$ by one-way analysis of variance (ANOVA) testing and if they were also out of the range of gastrin values observed in uninfected postprandial rabbits. The "Microstat I" stastical package[18]

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[17]Sigma Cell Culture Reagents; St. Louis, MO 63178

[18]Ecosoft, Inc., Indianapolis, IN 46220

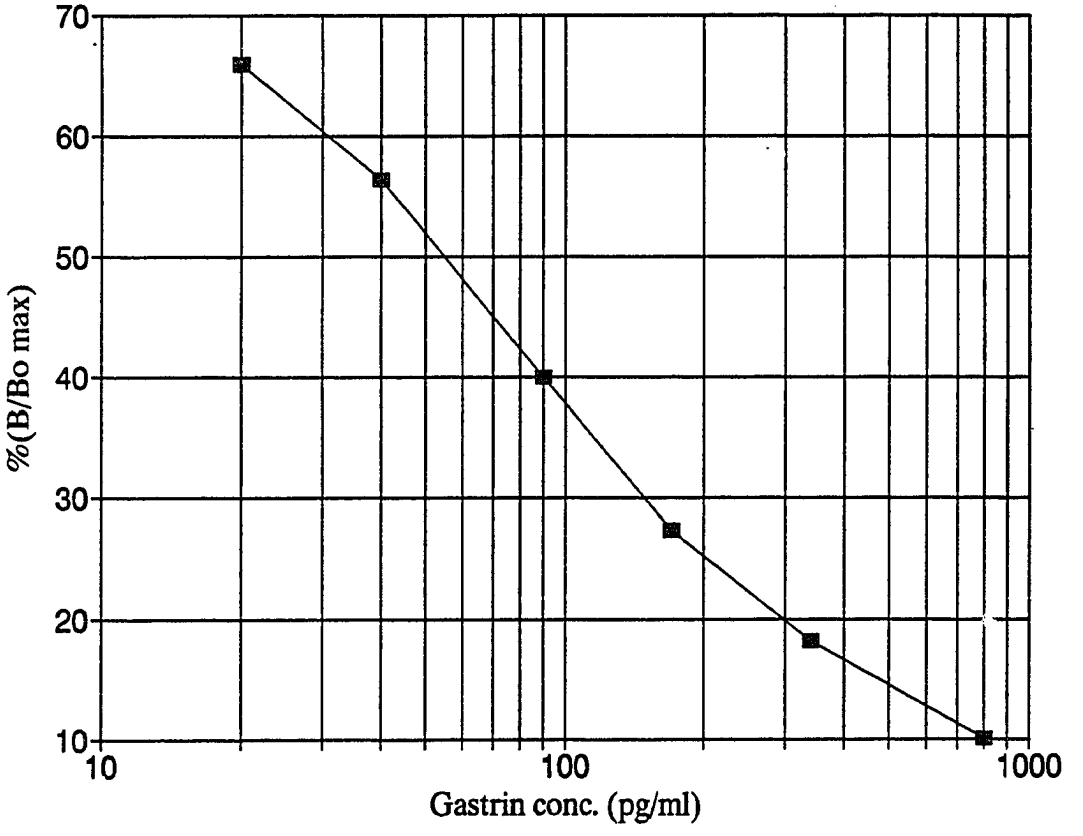

Figure 3.1 Example of a standard curve for determination of gastrin concentration in rabbit sera: values for kit-supplied gastrin standards, at concentrations of 20, 40, 90, 170, 340, and 800 pg/ml, are plotted as percentages of the average B_o (maximum binding) for assay #6, and points are joined by a curve drawn free-hand.

was used for initial one-way ANOVA testing. Among-group variations significant at the $P < .05$ by ANOVA were then further analyzed by the "Duncan's New Multiple Range Test"[19], enabling multiple comparisons among sample means (Ott, 1977).

RESULTS

The serum gastrin values (pg/ml) for the control, primary-infection, and secondary-infection groups, listed by post-inoculation week (PIW), are presented in Table 3.1. (For comparing the primary-infection and secondary-infection groups, the data for the former group's PIW 1 and 2, and for PIW 35 through 41, were added together to form the subcategories as noted in Table 3.1.) Figure 3.2 illustrates the same data graphically.

Normal serum gastrin

The average serum gastrin concentration for postprandial control rabbits was 65 pg/ml (SD 39.0), with a relatively large range of normal values: 23 to 155 pg/ml. Rabbits which habitually consumed little of their daily ration by the afternoon sampling time tended to have the lowest values. Two of the 15 control values exceeded 95 pg/ml; at least one of these was for a rabbit which had consumed his entire (145 g) daily ration in the 5 hours immediately preceding the sample collection.

Serum gastrin in infected rabbits

The serum gastrin concentrations observed in primary-infection group during PIW 6 and 7, 176 and 177 pg/ml respectively, significantly exceeded the control group value ($P < .005$) and were beyond the broad range of normal values. In the secondary-infection group, serum gastrin values never significantly exceeded the control group value, although concentrations observed during PIW 6 and 7 were significantly ($P < .05$) higher than values found in the early (PIW 1 & 2) and late (PIW 39 - 45) weeks of observation for this group.

When the two inoculated groups were compared on a week-by-week basis, the gastrin values

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[19]D. Reyna, copyright 1983

TABLE 3.1. SERUM GASTRIN CONCENTRATIONS IN OBELISCOIDES-INFECTED NEW ZEALAND WHITE RABBITS, BY GROUP AND POST-INOCULATION WEEK (PIW)

PIW interval	n	gastrin (pg/ml) mean	SE	range	super-scripts*
Control	15	65	10.1	23 - 155	
Primary infection					
1	7	59	30.4	12 - 240	
2	8	38	10.1	8 - 85	
subgroup					
1 & 2	15	48	14.8	8 - 240	(a)
3	9	141	47.4	59 - 490	
4	5	102	37.8	37 - 250	
5	2	63	20.5	42 - 83	
6	4	176 **	19.9	125 - 220	(b)
7	5	177 **	16.6	135 - 210	(b)
8	4	150	10.6	120 - 170	(b)
10	4	122	11.9	92 - 150	(b)
11 - 15	8	89	13.0	41 - 140	
35	2	91	24.5	66 - 115	
37, 41	2	35	12.0	23 - 47	
subgroup					
35 - 41	4	63	19.6	23 - 115	
Secondary infection					
1 & 2	4	35	6.9	14 - 43	(a)
6	3	110	14.1	86 - 135	
7	3	116	37.0	74 - 190	(b)
8	3	100	20.2	79 - 140	
9 & 10	3	63	11.4	42 - 81	
11 - 15	3	70	10.8	49 - 85	
39 - 45	4	42	6.9	29 - 61	(a)

* Groups with different superscript letters are significantly different from each other at $P < .05$

** These groups differ significantly from the control group at $P < .005$

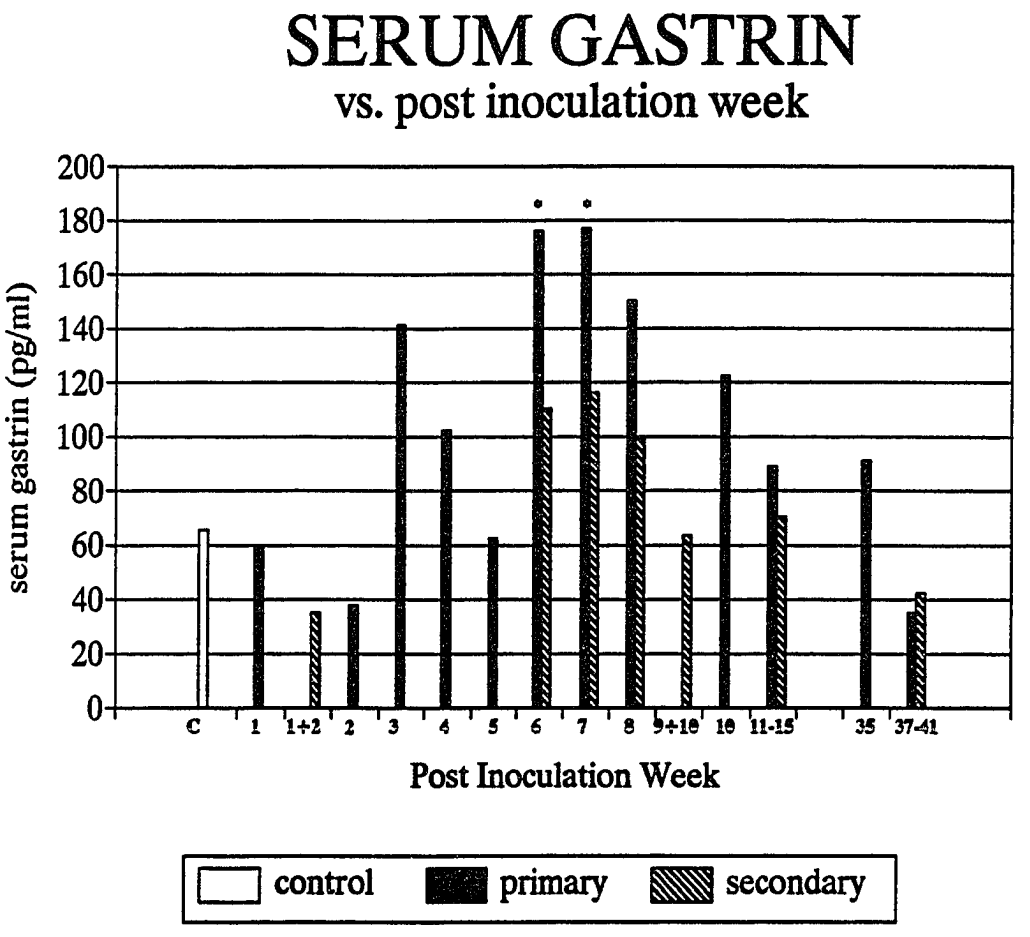

Figure 3.2 Serum gastrin concentrations (pg/ml) for control (uninfected) rabbits, and for rabbits with primary and secondary Obeliscoides infections at various intervals post-infection (post-inoculation week, PIW). The concentrations of groups marked (*) are significantly different from the control group at P<.005.

observed for PIW 6 and 7 during primary infections significantly exceeded all the other gastrin concentrations observed in this group except for the adjacent intervals PIW 8 and 10. These primary-infection group values also exceeded all of gastrin values observed in the secondary-infection group with the single exception of PIW 7.

Clinical signs

Clinical signs of gastrointestinal disturbance that were attributed to trichostrongylid parasitism included anorexia, depression, and, occasionally, a failure to reingest all cecoliths. Of the 20 infections observed in 17 rabbits (including 3 rabbits in which both primary and secondary infections were followed) over the total 45-week course of this study, 10 infections were associated with these clinical signs. In all 10 cases, the signs occurred within 3 weeks of larval inoculation. All infected rabbits were clinically normal during the remaining weeks, including PIW 6 and 7. All consumed the same quantity of pelleted ration at approximately the same rate as they had in the month prior to infection.

Of the 10 infections associated with anorexia during the first 3 post-inoculation weeks, 8 were among the 13 in the primary-inoculation group. Their anorexia was moderate to marked for as few as 3 to as long as 16 days within the interval PID 2 to 20 (PIW 1 to 3). In the secondary-infection group, clinical signs were less frequent, earlier, and briefer than in the primary infection group. Only 2 of 7 rabbits in the secondary-infection group became moderately to markedly anorectic. In both cases these signs began within a day of larval inoculation, and were no longer apparent by the end of PIW 1.

Unfortunately, serum samples within the first 3 post-inoculation weeks were available for only 5 of the 8 anorectic rabbits in the primary-infection group, and from neither of the 2 anorectic rabbits in the secondary-infection group). These sera, as well as all sera from the secondary-infection group collected during PIW 3, 4, and 5 were lost due to a malfunctioning freezer.

Parasitological findings

Parasite maturation and fecundity as determined by fecal flotation examinations during the study indicated that 10 of the 13 primary and 2 of the 7 secondary inoculations resulted in maturation

and reproduction by Obeliscoides. For the primary infections, prepatent periods ranged from 13 to 48 days, with patency usually beginning during PIW 3. Peak egg production levels ranged from 1 to 243 eggs per gram (epg), and were reached between PIW 3 and 11. The patent period ranged from 5 to 150 days (during PIW 3 to 27). For the two patent secondary infections, prepatent periods were 27 and 34 days (PIW 4 and 5), peak egg production levels of 175 and 241 epg were reached during PIW 8 and 10, and patency lasted 89 and 102 days (at PIW 18 and 19).

Of the 12 patent infections, egg production was followed through the interval between PIW 6 and 10 for 8 rabbits, including 6 primary and 2 secondary infections (4 rabbits were necropsied prior to that time). In all 8 cases, this interval coincided with the patent period. Among the 7 primary infections followed through patency, both the size of the larval inoculum and length of time it was refrigerated had an influence on the patency which resulted. All 3 of the patencies shorter than 35 days were established by smaller doses (1000 to 2500 L3) of Lepus-origin larvae which had been refrigerated less than 2 weeks. All 4 of the patencies lasting longer than 8 weeks were established by larger doses (16,300 to 31,400 L3) of rabbit-origin larvae which had been refrigerated as long as 22 weeks. Further information on patterns of trichostrongylid egg production in the colony is presented elsewhere (Nielsen, Chap. 1).

Of the 13 primary-infection group rabbits, 10 were necropsied and 3 were re-inoculated with infective larvae, to be later necropsied in the secondary-infection group. At the time of necropsy, lesions were classified histopathologically as "severe" for 7 of the 10 primary infections, but were "moderate" or "mild" for 6 of the 7 secondary infections (Nielsen, Chap. 1). Immature or adult Obeliscoides were present on or in the mucosa in 9 of the 10 primary infections, including between 900 and 43,000 largely immature Obeliscoides in 5 rabbits necropsied prior to PIW 12. The other 5 rabbits, necropsied between PIW 14 and 41, were not patent at the time of necropsy, but 4 of them had between 24 and 3700 apparently sterile Obeliscoides within the stomach. Serum gastrin concentrations at necropsy averaged 72 pg/ml (range 42 - 98 pg/ml, SD 23.4) for the former group, and 36 pg/ml (range 16 - 54 pg/ml, SD 16.1) for the latter group.

In the 7 secondary-infection rabbits, the 2 patent infections had ceased detectible egg production 21 and 26 weeks prior to necropsy. Nevertheless, between 10 and 450 stunted Obeliscoides were found on the gastric mucosa of all 7 rabbits. For the 3 rabbits necropsied prior to PIW 12, serum gastrin concentrations averaged 69 pg/ml (range 42 - 85, SD 23.7); for the 4 rabbits

necropsied after PIW 14, serum gastrin concentrations averaged 42 pg/ml (range 15 - 61, SD 19.6).

DISCUSSION

Radioimmunoassay technique

With two modifications, the commercial RIA kit used in this study, which was designed to measure human gastrin, provided a simple, accurate, relatively inexpensive means of measuring serum gastrin in small volumes of rabbit serum. The study demonstrated that the use of rabbit-origin antibodies to measure substances within rabbit serum is feasible if charcoal-precipitation techniques (Hansky and Soveny, 1977) are used to precipitate the bound antibody complex. The first incubation time was also increased, to 46 hours, to allow for equilibrium antibody binding (Walsh, 1974; Hansky and Soveny, 1977; Jaffee and Walsh, 1979; Wolfe et al, 1985).

Gastrin appeared to be stable within rabbit serum in spite of prolonged low-temperature storage, as has been observed for other species (Hansky and Soveny, 1977; Anderson et al, 1981; McGuigan and Wolfe, 1982; Entrocasso et al, 1986; Fox et al, 1987; Snider et al, 1988). The hormone was unstable, however, in a large group of samples which thawed for an unknown period (up to 3 days) during a freezer malfunction, despite the apparent short-term heat-stability reported previously (Yalow and Berson, 1970; Warburton and Close, 1987).

Normal serum gastrin concentration

Serum gastrin levels observed for normal rabbits in this study (65 ±10.1 pg/ml) were similar to values observed in other species. For humans, from which fasting samples can be relatively easily, stresslessly obtained, "basal" gastrin values are generally less than 75 pg/ml (Yalow and Berson, 1970), while postprandial samples reach 120 pg/ml (Blair et al, 1987). Normal fasting human values for the commercial kit used in this study were given by the manufacturer as 73 pg/ml, with a range for 60 normal subjects of between 48 and 116 pg/ml.

Fasting gastrin values in other monogastric species (dogs, cats, rats) are generally below 100 pg/ml (Dockray, 1975; Thompson, Rayford et al, 1975; Albinus et al, 1976; Castro, Copeland et al,

1976; Johnson, 1975). The single published report on rabbit gastrin presents a chart with 10 concentration points ranging from 90 to 300 pg/ml (Chey, Tai, et al, 1975), with no information on if these rabbits were fasted. The present results, even though they are for postprandial rabbits, are considerably lower than these observations, while the higher values reported by Chey et al (1975) exceed those found in this study among Obeliscoides-infected rabbits.

Unfortunately, in many of the published studies on animal gastrin concentrations, the blood sampling criteria are not given in the context of the subjects' usual feeding cycle. The various molecular forms of gastrin have relatively short half-lives, probably less than 30 minutes (Walsh, 1975) and are therefore liable to fluctuate with a variety of endogenous and exogenous stimuli. In addition, gastrin values have also been reported to fluctuate in circadian rhythms in both humans (Kanabrocki et al, 1985; Mazzetti di Pietralata et al, 1987) and rodents (Oscarson et al, 1979; Pasley et al, 1987). Experimental designs that disrupt normal circadian feeding cycles may yield ambiguous and misleading gut hormone concentrations (Rubin et al, 1986). To minimize some of these variables, serum sampling in the present study was maintained at a relatively constant time (1.5 to 5.5 h post-feeding) within the regular colony husbandry schedule.

Gastrin is an essential hormone for triggering the release of acid by gastric parietal cells, and its blood levels are regulated by gastric antral pH in a simple negative feedback loop (Walsh and Grossman, 1975; Titchen and Anderson, 1977). In vertebrates, the primary physiologic stimuli increasing serum gastrin concentration above basal levels are increased gastric fill and the presence of peptides/amino acids in the antral lumen (Walsh and Grossman, 1975; Yau, 1982). Acid production by isolated gastric glands, and its stimulation by pentagastrin, has been previously studied in rabbits (Berglindh et al, 1976b).

In the normal rabbit, gastrin increases are probably related primarily to both food consumption and to cecotrophy. Since all serum samples were postprandial, individual variations in the amount of daily ration consumed immediately after food was offered is probably one reason for the rather wide range of gastrin concentrations observed in uninfected rabbits: 23 - 155 pg/ml. There were indications that gastrin values within this range varied directly with the volume of recently ingested commercial ration present within the stomach at the time of sampling.

Nevertheless, postprandial serum sampling was selected in preference to "fasted " sampling

for this study because the stomach of a physiologically normal rabbit is probably never "empty" (Berglindh et al, 1976a; Cheeke, 1987). Several investigators (Laplace et al, 1974; Laplace et al, 1975; Henning and Hird, 1972) have discussed the normal balance which lagomorphs maintain between gastric and cecal fill. This results in long apparent retention times for even the fiber component of the ingesta, as verified in a related aspect of the present study (Nielsen and Holleman, 1989; Nielsen, Chap. 4). It has also been reported that, compared to control stomachs, 50% of the dry weight content remains in the stomachs of rabbits after a 24-hour fast in which cecotrophy is prevented (Carmichael et al, 1945).

The rate of food consumption may be a additional factor influencing the serum gastrin concentrations observed in this study. Rabbits regularly maintain a very low gastric pH (Smith, 1965). The slow, bit-by-bit consumption of commercial diet stretched over a period of 12 hours, which was the normal eating pattern for certain individuals in this study, may require only small increases in gastrin above the basal level to maintain acidity. Conversely, the rapid consumption of the same amount of diet compressed into a 3- to 5-hour span, the normal eating pattern for other individual rabbits, would require larger increases in gastrin to maintain acidity. Since the serum sampling time was set at between 2 and 5 hours after feeding, and given the short half-life of gastrin in serum (Walsh, 1975), relatively large differences in serum gastrin could have resulted even among normal individuals which initially ate at different rates, although they may have eventually consumed the same amount of food per day.

Individual variations in timing and amount of cecotrophy prior to serum sampling may also directly affect measured gastrin concentrations, irrespective of when commercial rations were first offered. Although feeding time (and daily light cycle) was held constant, each individual selected its own pattern of ration ingestion vs. cecotrophy. Previous studies have demonstrated that cecotrophy usually begins 6 to 8 hours after the maximum of feed consumption, and that some animals have more than one cecotrophic periods (Jilge, 1974; Hornicke, 1981). In this study, cecotrophy was not restricted to the dark period, and occasionally overlapped the consumption of the pelleted ration in some rabbits.

The particular effects of cecotrophy on gastrin production are unknown. There are two potential arguments in favor of cecotrophy increasing serum gastrin concentration. The first is that fundic pH is known to increase from 2 to 5.1 in the first few hours of cecotrophy, when cecotrophs

with an internal pH of 6 to 6.5 essentially fill the fundus (Griffiths and Davies, 1963). Second, protein-rich meals are known to increase serum gastrin concentration in many species (Walsh and Grossman, 1975). In this case, the protein content of the commercial ration was about 14 %, while the protein content of cecoliths is about 28% (Cheeke, 1987). Both of these factors would suggest that cecotrophy could stimulate gastrin release.

However, two additional findings presented by Griffiths and Davies (1963) suggest that cecotrophy may actually have little effect on basal serum gastrin concentration. First, the protein-rich, relatively alkaline content of cecoliths is effectively insulated from the gastric lumen by a tough membrane enclosing living bacteria as well as high levels of phosphate buffer for as long as 6 hours post-ingestion. Second, the acidity of the pyloric portion of the stomach, the anatomical location of the pH-sensitive G cells which produce gastrin (Larrson et al, 1975), remains at a pH of 2.3 during cecotrophy. This latter observation agrees with Smith's (1965) findings, in which gastric pH remained well below 3.0 throughout the cecotrophic period.

If both food ingestion and cecotrophy were to stimulate gastrin secretion, then diurnal fluctuations in serum levels should be minimal in rabbits. The wide range of normal gastrin levels and the apparent postprandial increase in this hormone observed in the present study tends to support the suggestion of Griffiths and Davies (1963) that cecotrophy has little effect on basal serum gastrin concentration.

Serum gastrin in infected rabbits

In the present study, clinical signs were less frequent and occurred earlier in rabbits previously exposed to Obeliscoides. Similarly, clinical signs of secondary Ostertagia infections are earlier, briefer, or absent in previously exposed ruminants (Anderson et al, 1976; Pitt et al, 1988; Ploeger, Kloosterman, Borgsteede and Eysker, 1990).

Serum gastrin concentrations significantly increased during PIW 6 and 7 in rabbits with primary Obeliscoides infections. There was some indication of increased gastrin concentrations in later weeks, but results were not statistically significant. Significantly increased serum gastrin was not associated with clinical signs in infected rabbit hosts. Anorexia, the main sign, was observed in some of the rabbits within 3 weeks of larval inoculation, but serum gastrin concentrations were not

elevated. Similarly, Yang et al (1990) found that pigs given a single large dose of Ascaris suum failed to demonstrate any increases in serum gastrin to 32 days post-inoculation, despite the development of mild clinical signs within 2 weeks of infection. In contrast, significant elevations of serum gastrin were associated with the development of anorexia and other clinical signs during PIW 5 in Ostertagia ostertagi-infected cattle (Fox, Gerrelli, Pitt, Jacobs, Gill and Simmonds, 1989).

In the present study, the timing of significant elevations in serum gastrin levels were more closely associated with Obeliscoides fertility and with the character of pathological changes within the gastric mucosa than with clinical signs in the host. In primary infections, patency began between PIW 3 and 5, and egg production levels peaked between PIW 4 and 10. Therefore, increased serum gastrin concentrations were generally related to the early, increasing phases of the parasite's patency. Gastrin levels tended to be higher prior to PIW 12, when more fertile adult Obeliscoides were found in the stomachs of necropsied rabbits. Gastrin levels tended to be lower after PIW 15, when the Obeliscoides population found at necropsy consisted largely of immature, stunted, and apparently infertile nematodes. Because serum gastrin levels remained low despite the persisting presence of infertile trichostrongylids, it appeared that significant increases occurred only in association with the achievement of fertile maturity by Obeliscoides.

The present isolate was characterized by the production of severe gastric mucosal lesions in primary Obeliscoides infections necropsied prior to PIW 15, and more moderate, chronic mucosal lesions after that time and in secondary infections (Nielsen, Chap. 1). Increased mucosal thickness was especially prominent in the former group, and generally coincided with both patency and higher gastrin concentrations. A similar relationship between increased mucosal thickness and serum gastrin immunoreactivity has been reported in Ostertagia ostertagi-infected cattle (Snider et al, 1988). In the present study, the chronic mucosal lesions were associated with persisting populations of immature or infertile Obeliscoides and generally low serum gastrin concentrations. These lesions resemble those reported for nonpermissive Ost. ostertagi infections in rabbits and pre-type II ostertagiasis in ruminants, in which gastric trichostrongylids were not fertile (Snider et al, 1981, 1985).

Increased serum gastrin levels in ruminants with primary Ostertagia spp. infections reportedly coincide with trichostrongylid maturation, as determined by the appearance of patency, changes in gastric pH, or pepsinogen increases (Anderson et al, 1976; 1981; Blanchard and Wescott, 1985; Entrocasso et al, 1986; McKellar et al, 1987; Fox et al, 1987; Fox, Gerrelli, Pitt, Jacobs, Gill and

Simmonds, 1989). When adult Ostertagia were transferred directly from one host to another in 3 studies, serum gastrin levels increased in the recipient host within a week after the transfer (Anderson et al, 1985; Titchen, 1982; McKellar, 1987). Transplanted adult Ostertagia can exert relatively immediate effects on gastric pH or serum pepsinogen (Eiler, 1981; Titchen, 1982; Anderson et al, 1985; McKellar et al, 1986; 1987). Significant elevations may continue for 10 to 20 days in primary infections established by a single transfer of adult parasites (McKellar et al, 1987).

The post-inoculation timing of the gastrin increase observed in the present Obeliscoides study, PIW 6 and 7, corresponds to that reported for primary ruminant Ostertagia infections established by infective larvae, between PIW 4 and 7 (Reynolds et al, 1979; Anderson et al, 1976, 1981; Blanchard and Wescott, 1985; Fox et al, 1987; Fox, Gerrelli, Pitt, Jacobs, Gill and Simmonds, 1989). Single infective doses were used in only 3 of these 7 studies (Blanchard and Wescott, 1985; Fox et al, 1987, 1988); in the remaining publications multiple doses of infective larvae were given after initial inoculation on PID 0.

In the present study, serum gastrin concentrations were consistently lower in rabbits experiencing a secondary Obeliscoides infection. Clinical signs were also less frequent and occurred earlier in these rabbits, and only 2 of these infections subsequently achieved patency, during PIW 4 and 5. Nevertheless, all 7 rabbits harbored immature, stunted or infertile trichostrongylids at necropsy. This is consistent with reductions in the maturation and fertility of Obeliscoides in previously infected rabbits reported by Michel et al (1975) and Fox (1976). Similarly, secondary Ostertagia infections in ruminants are notable for their earlier, briefer, or absent clinical signs and for their low patency (Anderson et al, 1976; Armour et al, 1979; Pitt et al, 1988; Jacobs et al, 1989; Ploeger, Kloosterman and Borgsteede, 1990a).

Despite the general absence of fertility in secondary Obeliscoides infections, gastrin levels exceeded the normal range during PIW 7, although this increase was not statistically significant. (It is unfortunate that sera collected from secondary infections during PIW 3 through 5 were accidentally lost, so that the commencement point of this possible increase and its duration prior to PIW 7 are unknown.) Mild increases in serum gastrin levels have similarly been observed in cattle and sheep with low-patency secondary trichostrongylid infections (Anderson et al, 1981; Entrocasso et al, 1986; Jacobs et al, 1989). Another cattle study failed to detect gastrin increases, but it terminated at PIW 6 (Pitt et al, 1989). Increased serum pepsinogen concentrations also accompany secondary ruminant

Ostertagia infections, suggesting that physiological disruptions continue to occur in apparently immune hosts harboring few fertile trichostrongylids (Armour, 1979; Kerboeuf et al, 1981; Entrocasso et al, 1986; McKellar et al, 1986; Wiggins and Gibbs, 1987, 1990; Pitt et al, 1988).

Conclusions

Titchen (1982) discusses the hypothesis that trichostrongylid parasitism is associated with a biphasic gastrin increase: an early rise associated directly with the presence of adult parasites on the mucosa, and a later increase associated with rising lumenal pH. The implication of this hypothesis is that the early increase would be observed in both naive and immune hosts, coinciding with parasite maturation, but any later increases would be seen only in naive hosts in which larger or more prolific infections increase lumenal pH. Observations in the present study would support this hypothesis: an early increase in serum gastrin was apparently associated with maturation and fertility among Obeliscoides, and coincided with prominent hyperplastic mucosal lesions. The later increase was not apparent, however, because persistent infections of this isolate were largely infertile, mucosal lesions were less severe, and gastric pH was unchanged (Nielsen, Chap. 1).

The use of RIA techniques for serial serum gastrin evaluations proved a relatively simple means of evaluating serum gastrin concentrations in the laboratory rabbit. Further studies of this economical host/parasite model system could easily incorporate serial gastrin determinations as a tool to further the understanding of the diverse physiological changes associated with both acute and chronic gastric trichostrongylid infections.

CHAPTER 4

EFFECTS OF GASTRIC TRICHOSTRONGYLID INFECTIONS ON GASTROINTESTINAL NUTRIENT CONTENT, EXCRETION, AND ON TRANSIT IN RABBITS

INTRODUCTION

Trichostrongylid nematodes, common inhabitants of the gastrointestinal (GI) tract of ruminants, cause economically important losses of productivity (meat, milk, wool, etc.) in the absence of clinical signs. For intestinal trichostrongylids, the metabolic basis of these losses was initially clarified in a series of elegant experiments utilizing a rodent model (Symons and Jones, 1971, 1972, 1978a, b; Symons et al, 1974). In sheep, intestinal trichostrongylids decreased apparent protein digestibility and enhanced endogenous N loss, with major deleterious effects on productivity in most studies (Sykes and Coop, 1976; Roseby, 1977; Holmes, 1985; Poppi et al, 1986; Symons, 1989). Some investigators also found effects on mineral utilization and gastrointestinal passage of ingesta (Roseby, 1977; Wilson and Field, 1983; Sykes, 1982, 1987; Holmes, 1985).

The physiologic basis for losses associated with abomasal trichostrongylid genera, however, is less clear than for the intestinal genera, although long-term nutritional effects are known to be substantial (Sykes and Coop, 1977; Parkins et al, 1982a, b; Holmes, 1985; Symons, 1989). Various species of the important genus Ostertagia constitute the most common abomasal parasites of cattle and sheep in northern temperate and subarctic climates, but numerous attempts at long-term passage of ruminant-origin abomasal trichostrongylids in small laboratory rodents have largely failed (Mapes and Gallie, 1977; Zebrowska-Plata, 1980; Snider et al, 1985; Court et al, 1988; Okamoto et al, 1988; Wagland et al, 1989; Adams, 1990; Conder et al, 1990). Although Trichostrongylus axei of bovine abomasal origin has been successfully passaged in the stomachs of rabbits for more than 25 years (Lyons et al, 1987), the pathogenic effects of these infections are substantially less than those of Ostertagia (Symons, 1989).

The genus Obeliscoides, including gastric trichostrongylids naturally occurring in lagomorphs (Measures and Anderson, 1983a; Fukumoto, 1986), is taxonomically close to Ostertagia, has been successfully passaged in laboratory rabbits, and has been proposed as a model for ruminant Ostertagia spp. infections (Worley, 1963; Russell et al, 1966; Sollod et al, 1968; Sollod and Allen, 1971). Only a single study on the nutritional effects of Obl. cuniculi in rabbits has been reported (Pace and Frandsen, 1982), but its results substantiated the potential utility of this system as an Ostertagia model. Rabbits dosed with high numbers (30,000) of infective third-stage larvae (L3) utilized feed less efficiently compared to uninfected rabbits, however in rabbits receiving "low levels" (1800 L3) of larvae the apparent digestibilities of protein, organic matter and ash were actually enhanced (Pace and Frandsen, 1982). Assessment of the nutritional effects of infection in that study were limited to the early post-inoculation period (21 days) and were complicated by feed ration differences.

In ruminants, Ostertagia infections cause both functional GI disruptions and reduced utilization of important nutrients. Decreased protein or N digestibility has been demonstrated in both cattle and sheep, although it is often difficult to separate from the anorexia observed in many of these infections (Sykes and Coop, 1977; Randall and Gibbs, 1981; Parkins et al, 1982a,b; Fox, Gerrelli, Pitt, Jacobs, Gill and Gale, 1989). Alterations in post-absorptive N metabolism have been also been demonstrated in ruminant Ostertagia infections (Steel, 1974; Steel and Symons, 1982; Topps, 1983; Symons, 1989). The effects of GI parasitism on protein metabolism are complex, systemic, and may be related to immunological and endocrine effects (Topps, 1983; Holmes, 1985; Symons, 1989). Even within the GI tract, increases in fecal N excretion do not inevitably accompany Ostertagia spp. infections because of alternative routes of loss and the potential for compensatory resorption by the distal GI tract (Sykes, 1982; Steel and Symons, 1982; Symons, 1989).

Increased fecal N and Ca losses have been associated with Ostertagia infections in ruminants, but it is frequently difficult to determine if these are the result of reduced absorption or increased, uncompensated excretion of endogenous or exogenous origin, or if both absorption and post-absorptive metabolic processes are disrupted (Steel and Symons, 1982; Sykes, 1982; Wilson and Field, 1983; Symons, 1989). While intestinal trichostrongylids affect the absorption of P and perhaps Ca (Wilson and Field, 1983; Poppi et al, 1985) as well as other minerals and ions (Sykes, 1982; Steel and Symons, 1982; Symons, 1989), Ostertagia infections have only been shown to reduce apparent Ca absorption (Wilson and Field, 1983). While Ostertagia may have caused an observed reduction

in serum Ca during post-infection weeks (PIW) 5-8 (Fox, Gerrelli, Pitt, Jacobs, Gill and Simmonds, 1989) and substantial reductions in skeletal Ca content at PIW 14 (Sykes and Coop, 1977), many investigators have ascribed skeletal effects to metabolic or protein effects rather than absorptive changes (Sykes, 1982, 1987). A reduction in protein availability could reduce intestinal Ca absorption and serum Ca levels (Kenney, 1981; Fox et al, 1989b), but of particular interest is the possible alteration of Ca absorption patterns associated with the persisting alkaline shift in abomasal pH often observed in ruminant Ostertagia infections (Titchen, 1982; Holmes, 1985; Symons, 1989). In both ruminants and laboratory rodents, the duodenum and anterior small intestine are the primary absorption sites for Ca (Waldron-Edward et al, 1966; Bivin et al, 1979; Scott and MacLean, 1981; White et al, 1984; Hove et al, 1986; Shiga and Morina, 1986; Staaland et al, 1986; Cheeke, 1987), and substantial gastric pH increases potentially shift the ingesta in these segments toward an increased alkalinity which is less conducive to absorption, thus enhancing the possibility of precipitation and fecal loss (Kenney, 1981; Shiga et al, 1987; Fox, Gerrelli, Pitt, Jacobs, Gill and Simmonds, 1989).

The absorptive capacity of each segment of the GI tract has an obvious dependence on the amount of time ingesta remain there (Waldron-Edward et al, 1966; Uden et al, 1982; Sakaguchi et al, 1987), and the more overt clinical effects of trichostrongylid infection (anorexia, diarrhea) may be related to disruptions of normal ingesta passage. While intestinal parasites have been associated with altered GI passage rates (Castro, Badial-Aceves et al, 1976; Bueno and Fioramonti, 1980; Symons, 1989), those sited in the stomach or abomasum have a unique potential to alter the main neuroendocrine mechanisms controlling pyloric relaxation, intestinal transit, and the release of enzyme-rich fluids within the entire lower GI tract. Reduced digesta flow and enhanced fluid loss have been described in sheep infected with the abomasal trichostrongylids Trichostrongylus axei or Haemonchus contortus (Bueno, Dakkak and Fioramonti, 1982; Bueno, Honde et al, 1982; Dakkak, 1984). Since gastrin has a variety of effects on motility and digestive processes (Barlet, 1973; Bell et al, 1977; Yau, 1982), and since increases in serum levels of this hormone often accompany ruminant and rabbit Ostertagia infections (Titchen, 1982; Holmes, 1985; Snider et al, 1985; Symons, 1989), disturbances in GI passage parameters associated with Ostertagia-induced hypergastrinemia have been postulated (Titchen, 1982; Symons, 1989). A prolongation of passage rate temporally associated with peak serum gastrin levels was recently demonstrated for anorectic calves infected with Ostertagia ostertagi (Fox, Gerrelli, Pitt, Jacobs, Gill and Gale, 1989).

Gastrin is a primary control for the rate of pyloric outflow into the duodenum (Bell et al, 1977; Yau, 1982). Although a variety of inert markers have been used to measure GI passage, ingesta flow has rarely been measured in this way for trichostrongylid-infected ruminants (Fox, Gerrelli, Pitt, Jacobs, Gill and Gale, 1989). Measurement of passage through only a part of the tract, as would be needed to determine pyloric outflow, has traditionally involved more intrusive techniques, including cannulation, radiology, or electromyography (Pickard and Stevens, 1972; Corpet and Laplace, 1976; Bjornhag and Sjoblom, 1977; Bueno and Fioramonti, 1980; Bueno, Dakkak and Fioramonti, 1982; Bueno, Honde et al, 1982; Ehrlein and Ruoff, 1982; Uden et al, 1982; Ehrlein et al, 1983; Gidenne et al, 1988). Recently, Sakaguchi et al (1987) have suggested that the use of regression equations to determine passage rate parameters from the excretion curves of single-dose radioisotopic (RI) markers might provide a nonintrusive, accurate estimate of pyloric outflow in rabbits.

Because mean retention times for large fibers are very short (16 to 38 hours) in rabbits, and because this feed component passes so rapidly through the GI tract after it leaves the stomach, Sakaguchi et al (1987) proposed that the rate constant characterizing their excretion could be regarded as a reflection of the gastric dilution of the marker. This suggests that determination of fiber passage rates for both trichostrongylid-infected and control rabbits would allow a comparison of their pyloric emptying rates, and the detection of infection-associated prolongation previously observed in ruminants (Bueno, Dakkak and Fioramonti, 1982; Fox, Gerrelli, Pitt, Jacobs, Gill and Gale, 1989).

Single dose RI marker techniques utilizing regression equations fitted statistically to the time-course decline of fecal marker concentration have been used in ruminants and rabbits to determine GI passage rate parameters (Brandt and Thacker, 1958; Uden et al, 1982; Sakaguchi et al, 1987; Holleman and White, 1989; Sakaguchi and Hume, 1990). Transit time, total mean retention time, and the rate constant of long-term marker excretion (k) have been determined by evaluation of the fecal marker excretion curve (Holleman and White, 1989). These authors also evaluate the turnover time within the slowest (rate-limiting) compartment, which is often equivalent to the rumen turnover time for ruminants (Holleman and White, 1989), but might more appropriately be called the GI turnover time for nonruminants. Separate radioisotopic (RI) markers may be used to obtain independent passage rate parameters for both fibrous and liquid components of the ingesta (Uden et al, 1982; Sakaguchi et al, 1987; Sakaguchi and Hume, 1990). Determination of the liquid component has proved particularly difficult in the rabbit because of the duality of its fecal excretion

(Corpet and Laplace, 1976; Uden et al, 1982; Sakaguchi et al, 1987; Sakaguchi and Hume, 1990). Regular cecotrophy and operation of a colonic separation mechanism result in the rapid passage of fibers larger than 350 microns, which are excreted with the regular feces, and the delayed passage and recycling of part of the nutrient-rich small particulate/liquid component, which appear as cecoliths destined for immediate reingestion (Bjornhag, 1972, 1987; Leng, 1977; Ehrlein et al, 1983; Sakaguchi et al, 1987).

Cecotrophy has 2 important potential effects on GI passage rate parameters. (1) Although lactate is the most abundant product of gastric cecotrophic digestion (Griffiths and Davies, 1963), VFA's are also present (Henning and Hird, 1972). While VFA's are rapidly absorbed by the stomach, any increase in their concentration within the proximal GI tract may delay pyloric outflow and prolong intestinal passage, as demonstrated in sheep (Gregory and Miller, 1989). (2) Reingested cecoliths may remain as morphologically intact units within the rabbit stomach for as long as 6 hours before beginning to dissolve (Griffiths and Davies, 1963). The regular re-entry of this relatively liquid (Henning and Hird, 1972; Cheeke, 1987) cecal-origin material into the proximal GI tract differentially prolongs the excretion of fluid markers, compared to that observed for rodents reingesting regular fecal pellets (Krawielitzki et al, 1987; Verschuren and Nugteren, 1989). As a result, total mean retention time and GI turnover time for liquids are very long and have not previously been determined for the rabbit (Uden et al, 1982; Sakaguchi et al, 1987; Sakaguchi and Hume, 1990). Use of the single-dose dual RI marker technique allowed the determination of all passage rate parameters for both fiber and liquid components in normal, unrestrained rabbits (Nielsen and Holleman, 1989).

The recent isolation of an Alaskan isolate of Obeliscoides sp. and its passage in laboratory rabbits (Nielsen, Chap. 1) provided an opportunity to investigate the effects of gastric trichostrongylid infections on GI absorption and passage. Changes in serum levels of gastrin and other constituents are reported separately (Nielsen, Chap. 2 and 3), as are biological characteristics of the infections (Nielsen, Chap. 1). Because of the tendency of this isolate to delay maturation, infected rabbits with occult infections were held for relatively long periods, allowing the serial evaluation of fecal excretion of nutrients. When rabbits were necropsied at specific times post-inoculation, their GI tracts were partitioned into sections for the comparative analysis of nutrient content. Finally, the adaptation of a single-dose RI marker technique to rabbits for the first time allowed comparisons of the GI flow rates of both fiber and fluid components at stages of both primary and secondary infections.

MATERIALS AND METHODS

Animals and husbandry

Standard random-bred New Zealand White rabbits were acquired as weanlings (6 to 7 weeks of age) from a commercial supplier[20] and were habituated to the colony schedule for at least one month prior to larval inoculation. During the 20-month span of the study, the 18 rabbits (8 males, 10 females) were held in the colony between 15 and 84 wk (average: 37.0 wk). Rabbits were inoculated with trichostrongylid larvae when between 10 and 54 wk of age (between 1.8 and 4.5 kg BW); at euthanasia, rabbits ranged from 22 to 91 wk in age and weighed 2.8 to 5.3 kg.

Euthanasia was accomplished by preliminary anaesthesia for intracardiac blood collection using xylazine (5 mg/kg BW), acepromazine (1.0 mg/kg BW) and ketamine (60 mg/kg BW) followed by the post-sampling intracardiac injection of concentrated pentobarbital (approximately 150 mg/kg BW). A single rabbit died spontaneously 17 days post-infection and was found to have pulmonary lesions consistent with Pasturella multocida pneumonia; this organism was isolated from lung tissue collected at necropsy. Pneumonic or other extra-gastric lesions were not encountered in the remaining rabbits at necropsy.

The rabbit colony was maintained separately within an approved facility, on a 12-hour light/dark cycle (7 am, 7 pm). Animals were individually housed in stainless steel cages with grilled floors, with grill spacing wide enough that formed pellets and most cecoliths dropped onto a holding screen below the floor. The screen functioned to separate fecal material from urine.

Feeding

Rabbits were fed daily at a set time (approximately 1000 h), when food was placed in stainless steel hoppers attached to each cage. All rabbits received 130 to 170 g (depending on age) of commercial pelleted rabbit chow[21] as their sole food source. This amount of food was entirely

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[20]R & R Rabbitry, Stanwood, WA 98292

[21]Purina Rabbit Chow Complete Blend, Purina Mills, Inc., St. Louis, MO 63166

consumed within 24 hours when rabbits were healthy, and was somewhat less than ad libitum consumption would have been.

If more than one-third of the pelleted ration remained in an individual's feed hopper after 24 hours (i.e. at the next scheduled feeding), the rabbit was considered anorectic. The residual chow was discarded after weighing, and fresh chow was provided in an amount slightly exceeding that which had been consumed the previous day, until the rabbit's consumption returned to the usual daily level.

Guaranteed analysis of the commercial chow listed crude protein not less than 14.0%, crude fiber not more than 20.0%, crude fat not less than 2.0% and ash not more than 10.0%. Replicate analysis of 8 samples of chow, performed as described below for fecal and GI content materials, demonstrated 95.22 ±0.97% (=SD) dry matter (DM) content, 2.77 ±0.11% N content, 0.51 ±0.05% P content, and 1.13 ±0.10% Ca content. (Intra-assay coefficients of variation were 1.02%, 3.89%, 9.70%, and 9.15%, respectively.)

Sulfaquinoxaline treatment

Nine of the 18 colony rabbits had patent coccidia (Eimeria spp.) upon receipt as weanlings, were treated with a 10 to 14-day course of oral sulfaquinoxaline (SQXN) in drinking water at a dosage of .05 to .15 g/kg BW, and tested repeatedly negative prior to admission to the main colony. On two occasions during the 20-month study, 5 of the 18 colony rabbits shed detectable oocysts, resulting in the prophylactic treatment of the entire colony. Of 204 fecal excretion samples analyzed, 68 were collected during routine SQXN treatment; of 5 GI passage trials in which a total of 40 rabbits were examined, 20 rabbits in 2 trials were treated with SQXN. None of the 18 rabbits in the present study had gross or histopathological lesions of either intestinal or hepatic coccidiosis at necropsy.

Trichostrongylids

Rabbits were inoculated with third-stage (L3) infective Obeliscoides sp. larvae either by oral dosing with 1 or 2 gelatin capsules into unanesthetized rabbits (4 cases plus 1 control) or by gastric intubation (size No. 5 French) into lightly anesthetized (acepromazine 1.0 mg/kg BW; ketamine 25

mg/kg BW). Infective larval dosages ranged from 500 to 50,400 L3. Control rabbits were untreated or were sham-inoculated with similar volumes of tap water. Infective larvae were obtained from 12- to 18-day old moist vermiculite cultures of the feces of patent snowshoe hares collected in Fairbanks, Alaska (3 cases), or with first- (4 cases), second- (8 cases), or third-passage larvae obtained from colony rabbits (Nielsen, Chap. 1). The number of infective larvae dosed ranged from 500 to 50,400 L3 (Appendix A). Number of larvae per infective dose or their passage number were not considered as factors in the present study. Numbers of mature and immature Obeliscoides recovered at necropsy were also not considered; in many cases fecal excretion and GI passage observations were separated from necropsy time by many weeks.

Patent gastrointestinal parasitism was monitored with regular fecal flotation examinations using Sheather's Sugar Solution (detection level: 0.5 eggs per gram feces). At necropsy, 90% of the gastric mucosa was digested to allow the recovery of immature Obeliscoides sp, while 90% of the gastric content was reserved for the isolation of adult trichostrongylids; both samples were preserved in 70% ethanol/5% formalin until examination (Nielsen, Chap. 1). Identification was not carried beyond the generic level for this study because of the substantial percentage of stunted and immature trichostrongylids isolated from these infections.

Treatment groups

Samples were divided into treatment groups according to the rabbits' previous experience of Obeliscoides infection, and subdivided by time since larval inoculation. "Control" samples were those collected from rabbits which were sham inoculated with tap water as noted, and which had no previous trichostrongylid infections at the time of collection. "Primary infection" samples were those collected from rabbits which had received a single dose of infective larvae at any time up to 45 wk previously, while "secondary infection" samples were those collected from rabbits which had received an additional larval dose between 16 and 44 weeks after a primary infection was initiated. The numbers of rabbits in each category changed as fecal samples were collected and GI passage rates were determined over the 20-month course of the study: control rabbits became primary infection rabbits upon larval inoculation, and primary infection rabbits later became secondarily infected. By the end of the study, 2 of the 18 rabbits had not received an infective larval dose and remained as controls, 11 had received a primary infection only, and 5 had received a secondary infection.

Time since larval inoculation was counted with "day 0" as the day of inoculation, the first 7 days following as "post-inoculation week" (PIW) 1, etc. Both primary and secondary infections were observed to necropsy at PIW 45.

Collection and analysis of fecal excretion samples

Fecal excretion data was based on analysis of 204 samples of regularly excreted fecal material, herein called "fecal pellets", collected from 18 rabbits within 12 hours of excretion. Fecal pellets without traces of urine contamination were removed from the under-cage mesh screen, labeled, and stored frozen in separate plastic bags for 15 to 30 months until analysis. Because samples were subjected to variable periods of drying within the cages prior to collection, and because some samples were initially freeze-dried, all analyses were done on a dry weight (DM) basis.

Because rabbits were not collared, cecoliths were normally reingested during the study period. However, 25 cecoliths were spontaneously dropped during the study period and were casually collected for nutrient analysis. These included 3 cecoliths from uninfected control rabbits, 14 from rabbits with primary infections and 8 from rabbits with secondary infections. These samples were evaluated separately from fecal pellet samples.

For each sample, between 3 and 5 grams (g) of material was oven-dried to constant weight at 105 degrees C (Wilson and Field, 1983; Shiga and Morino, 1986). Dried samples were subjected to wet acid ashing using a block digester yielding a reaction temperature of 333 degrees C. Total nitrogen (N) and phosphorus (P) were determined simultaneously by automated continuous flow methodology in an auto-analyzer II system (Isaac and Johnson, 1976; Technicon Industrial Methodology, 1976; Brundage et al, 1981). Total Ca was determined by atomic absorption spectrophotometry[22] in a lanthanum background against a prepared standard (Baker and Suhr, 1982).

For fecal pellet samples, intra-assay coefficients of variation for % DM, determined by replicating 3 fecal samples 4 to 7 times, averaged 2.65%. For %N, P, and Ca, all samples were

[22]Model 5000 Perkin-Elmer Atomic Absorption Spectophotometer; Perkin-Elmer Corp., Norwalk, CT 06859

duplicated (37 samples were duplicated twice) and the average value was used. Intra-assay coefficients of variation for the 3 replicated samples (8 to 14 values), yielded 8.04% for N, 8.22% for P, and 9.96% for Ca.

A Student's t-test of sample means was used to compare the 11 control fecal samples collected during oral SQXN administration with the 19 control samples collected in the absence of treatment. Because no significant difference was found between the nutrient content of these groups ($P > .15$), all comparisons between fecal nutrient content of control and infected groups were made without regard to SQXN administration. For the 30 fecal samples collected from uninfected rabbits, %N, %P and %Ca were all found to be normally distributed. CV's and the effect of SQXN administration were not tested for cecoliths because sample size was insufficient.

Collection and analysis of postmortem GI segment samples

Segmental analysis of GI ingesta was done for 14 of the 18 rabbits, including 1 uninfected control, 8 with primary infections, and 5 with secondary infections. Procedures for the nutrient analysis of ingesta within 7 different segments of the GI tract at necropsy was as described for fecal samples. Total GI pool size was the sum of the DM, N, Ca or P content of all 8 segments. The total GI pool size was compared with nutrient intake for each of these nutrients on the day of necropsy, when rabbits were offered their usual daily ration at the regular feeding time and were euthanized (protocol began) 3.5 to 4.0 hours later (1330 to 1400). All 18 rabbits were examined for trichostrongylids and for gastric and other gross and histopathologic lesions, as reported elsewhere (Nielsen, Chap. 1).

For nutrient content analysis of ingesta, the GI tract was divided into 8 segments between the stomach and the anus, with analysis performed separately for each segment. Anatomical boundaries delineating GI tract segments for analysis were: "stomach" from cardia to pylorus, "duodenum" to the caudal flexure, "ileum" delineated by its antimesenteric attachment and including the (3 cm) sacculus rotundus, the remainder of the small intestine considered "jejunum" and divided exactly in half as "anterior" and "posterior" jejunum, "cecum" from ileocecal valve to tip, "colon" from ileocecal valve (including a 4 to 6 cm long ampulla caecalis coli) to the termination of haustrae and taeniae coli, and "rectum" extending distally to the anus. Length was determined at necropsy for all segments except the stomach prior to emptying ingesta, with care taken to avoid stretching. Mean

GI segment lengths for the 14 rabbits measured are presented in Table 4.1. After length determination, wet weights were determined for the entire content of each segment, and all ingesta were preserved frozen (except that only 10% of the wet weight of gastric contents was retained) separately in glass screw-cap jars for 9 to 18 months until analysis.

Nutrient content in specified segments of the rabbit GI tract were compared as relative or absolute concentrations. For DM, relative concentrations were as % of total (wet) weight of the segment (in grams) that consisted of DM, while for N, Ca and P relative concentrations were expressed in terms of DM content for the segment (g or mmol/100 g DM). Absolute concentrations were expressed as amount of nutrient per meter of segment length (g or mmol/m segment length).

Determination of GI passage parameters

GI passage parameters were determined in 5 separate trials using radioisotopic markers (RI trials). Techniques for the determination of passage rates by single marker doses were as previously described (Holleman and White, 1989; Nielsen and Holleman, 1989). Briefly, two nonabsorbable isotopic markers were used. The liquid phase marker, Cr-51-EDTA was obtained from a commercial supplier[23], assayed, and administered as a dose of between 10 and 30 microcuries (diluted in 100 to 300 microliters of normal saline). The particulate phase marker, cerium-141, was prepared by binding to large fibers in the feed. A single batch of commercial pelleted rabbit feed, used for all trials, was prepared by grinding with mortar and pestle, suspending in tap water, and thorough washing in a standard testing sieve[24] to retain only those feed particles larger than 355 microns. Prior to each trial, cerium-141-chloride[25]was incubated with the water-suspended particles and stirred continuously for several hours prior to removal of the unbound isotope by 2 cycles of centrifugation and rinsing. Washed, labeled feed particles were dried and placed in pre-weighed gelatin capsules to yield dosage based on capsule weight. Ce-141 doses were 1 to 2 microcuries.

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[23]NEN Research Products, DuPont Co.; Boston, MA 02118

[24]No. 45 U.S.A. Standard Testing Sieve, Tyler equivalent 42-mesh; W.S. Tyler, Inc.; Mentor, OH 44060

[25]NEN Research Products, as above

TABLE 4.1. GASTROINTESTINAL SEGMENT LENGTH AND BODY WEIGHT FOR 14 MATURE NEW ZEALAND WHITE RABBITS#

Segment	Mean length (cm)	(SD)	Range
Duodenum	31.00	(4.71)	22 - 40
Jejunum (ant.+post.)	182.93	(19.13)	148 - 215
Ileum	35.71	(3.20)	30 - 41
Cecum	47.62	(3.50)	40 - 53
Colon	32.93	(5.06)	20 - 42
Rectum	112.62	(17.19)	84 - 143
Body weight (kg)	mean	(SD)	Range
	4.21	(0.42)	3.67 - 5.15

Six male and 8 female rabbits aged 28 to 91 weeks (mean 46 weeks), fed a daily ration of 145 g Purina Rabbit Chow Complete Blend

At the beginning of an RI trial, 2 to 3 gelatin capsules (size "0") were given orally to each restrained but unanesthetized rabbit. Markers were always administered to unfed rabbits near the normal colony feeding time (1000 h), and dosed rabbits were returned immediately to their cages and offered their usual daily ration. Feeding continued as usual throughout the duration of each trial.

The GI excretion patterns of both markers were determined by the systematic collection of fecal pellets (and cecoliths, as available) as soon as possible after depostition, with the time interval for each sample measured from the time of marker administration. In the first radioisotope (RI) trial, fecal collection terminated at 125 h post-administration. Because a substantial proportion of the liquid phase marker dose had not been excreted at this time due to cecotrophic recycling, in the 4 subsequent trials fecal collection was continued to 240 h. Over 5 trials, between 25 and 62 (average 45.4) timed fecal samples were collected per individual for marker assay. Each sample was stored frozen until the end of the trial, freeze-dried to constant dry weight, placed in separate counting vials and assayed in a dual-channel gamma counting system[26] together with lab standards of both isotopes as well as standards consisting of similar amounts of the labeled feed material (Ce-141) or of the dosing solution (Cr-51-EDTA). Each sample point was plotted as specific activity (cpm/g) relative to sample dried weight against elapsed time (since marker dosing), and identified as a regular fecal pellet or cecolith.

For both fiber (Ce-141) and liquid (Cr-51-EDTA) markers, 3 GI passage rate parameters were separately determined for: transit time (TT), total mean retention time (TMRT), and gastrointestinal turnover time (GITT). These parameters were determined by analysis of the marker excretion data using equations based on the Stewart-Hamilton approach, as previously described (Holleman and White, 1989). Figure 4.1 illustrates marker excretion curves for an uninfected rabbit, with marker concentration in feces (cpm/g DM) plotted against time since marker dosing. Since post-peak marker concentration declined almost linearly, a least-square regression line with slope "k" was fitted to represent the "terminal excretion component". There was an initial phase of rapid increase in marker concentration prior to the peak concentration, as previously noted (Brandt and Thacker, 1958; Uden et al, 1982b; Sakaguchi et al, 1987). Transit time (TT) was determined by noting the elapsed time post-dosing of the first appearance of marker in feces. Total mean retention

[26]1195 Series Automatic Gamma Counting System, Searle Analytic Inc., TM Analytic, Inc.; Elk Grove Village, IL 60007

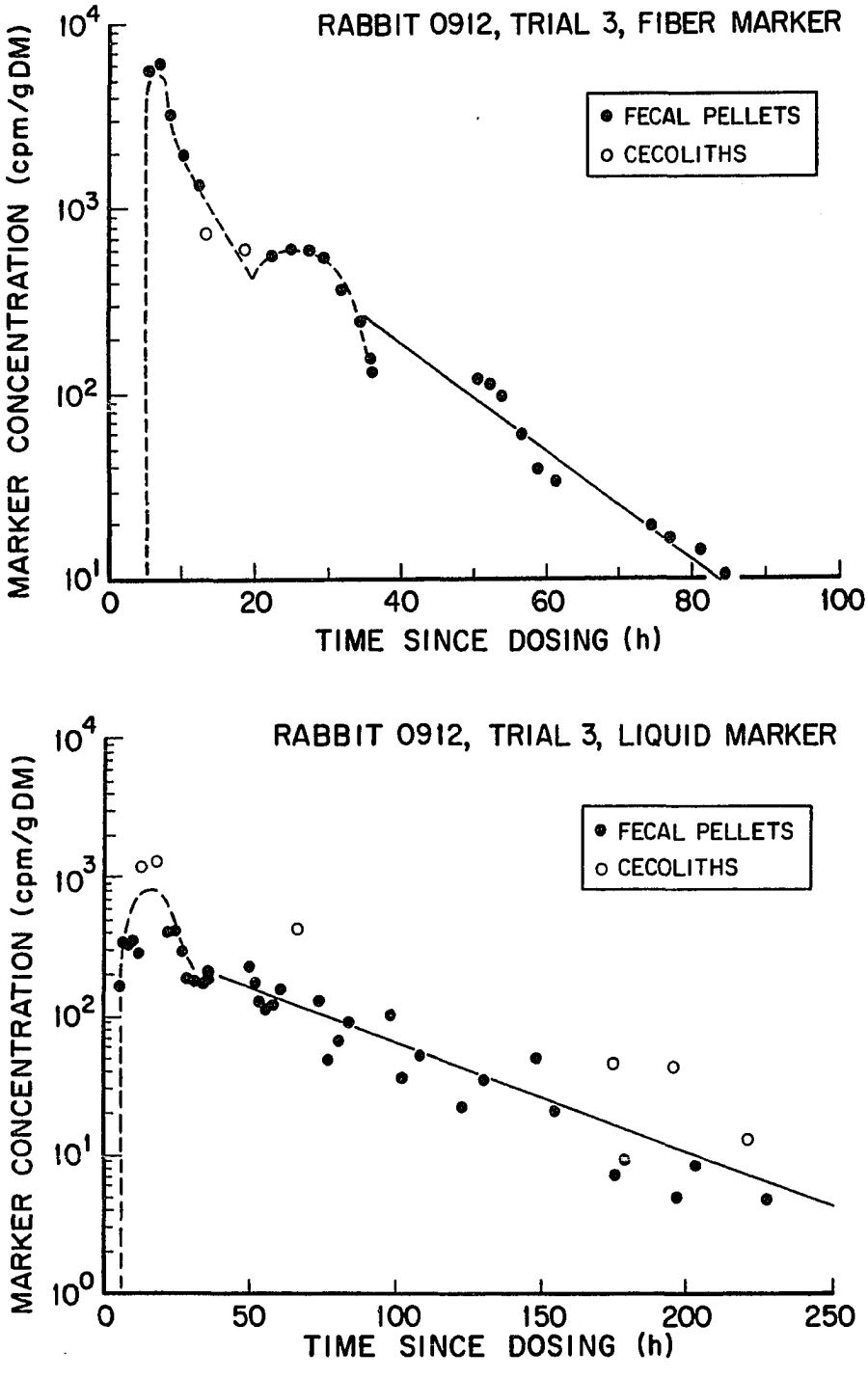

Figure 4.1 Control (uninfected) rabbit: Concentration of radioisotopic marker in feces (cpm/g DM) vs. time since dosing (h). Top: Concentration of the fiber-component marker, Ce-141; TT= 5.9, TMRT= 16.9 and GITT= 14.9 h. Bottom: Concentration of the liquid-component marker, Cr-51-EDTA; TT= 5.9, TMRT = 50.7 and GITT= 55.1 h. (Concentrations in regular fecal pellets are shown as plus signs, while those in cecoliths are shown as triangles.)

time (TMRT) was the cumulative time (including TT) spent in the GI tract by all of the marker material divided by the amount of marker (excreted dose). GI turnover time (GITT) was found as the reciprocal of the slope of the terminal excretion component line (1/k), and represented the turnover time of the rate-limiting part of the GI tract.

Dose was determined from the standards and from the amount of marker material given to each rabbit. To verify that the marker was not absorbed from the GI tract, 5-ml urine samples were assayed for each of the 12 individuals for the first 3 days of all trials, and no radioactivity was found.

In the first RI trial (RI-1), between 500 and 2500 infective larvae (L3) were used to establish Obeliscoides infections; in all subsequent trials more than 15,000 L3 were used (Appendix A). Also in RI-1, Elizabethan collars were used for 3 12-hour (overnight) intervals for 3 rabbits, but were generally unsuccessful in preventing cecotrophy, and furthermore caused stress and interfered with normal water consumption. Collars were not used in any of the subsequent trials. However, spontaneously dropped cecoliths were occasionally available for marker analysis from nearly all individuals. Cecolithic samples comprised an average of 18.32% (SD=10.46%; range 0 to 42.31%) of the total samples collected from each individual during the 5 trials.

Using 18 rabbits in 5 RI trials, GI passage rates were determined 40 times. Six rabbits were tested only once, 3 were tested twice, 8 were tested 3 times, and one rabbit was tested four times. If the potential effects of trichostrongylid infection status (tested below) was disregarded, inter-trial coefficients of variation (CV) for the 8 rabbits tested in three or more trials (excluding a single rabbit in which replications occurred during a secondary infection, PIW 16-26) were very high: only 6 of 48 were less than 10%. CV's also showed considerable differences depending on the passage parameter under consideration (Table 4.2). Within the same individual, TT of both fiber and liquid components tended to be the most variable, while GITT of the fiber component showed the least variation. Each of the passage rate parameters (TT, TMRT, GITT) for both component markers was normally distributed for the 11 uninfected rabbits.

Oral SQXN treatment coincided with RI trials 4 and 5, so that of the 40 passage rate determinations, 20 occurred during this treatment. The potential effects of oral SQXN on each of the 3 passage rate parameters were tested for 11 uninfected control rabbits, including 2 rabbits that were tested as uninfected both without and with treatment, using a Student's t-test with pooled

TABLE 4.2. VARIABILITY IN GI PASSAGE RATE PARAMETERS: COEFFICIENTS OF VARIATION (%)#

Passage Rate Parameter	mean	(SD)	Range
Fiber: TT	33.94	(22.74)	12.30 - 80.40
Fiber: TMRT	22.46	(9.21)	11.01 - 37.39
Fiber: GITT	11.26	(7.47)	4.09 - 26.71
Liquid: TT	38.24	(23.36)	12.43 - 80.40
Liquid: TMRT	14.97	(7.32)	3.55 - 27.54
Liquid: GITT	13.54	(7.54)	0.86 - 24.03

For 8 rabbits independently measured in 3 or more RI trials, disregarding Obeliscoides infection status. (No measurements of secondary infections during PIW 16-26.)

estimate of variance. In the 11 uninfected rabbits, all 3 passage rate parameters were normally distributed for both the fiber and liquid components.

Statistical analysis

Statistical comparisons were made among uninfected and infected rabbit groups by hypothesis testing of means and by one-way analysis of variance, as described below, using a statistical analysis package[27]. Differences were considered significant at $P<.05$. For 3 or more groups differing at $P<.05$ by ANOVA, Duncan's New Multiple Range Test was used for multiple comparisons between sample means (Ott, 1977; Steel and Torrie, 1980).

For both fecal excretion data and GI tract segment content at necropsy, %N, %P and %Ca were analyzed entirely separately. Similarly, fiber passage parameters (TT, TMRT and GITT) and those for liquid passage (TT, TMRT with SQXN, TMRT without SQXN, and GITT) were analyzed separately. One-way ANOVA was tested separately for control with primary infection groups and for control with secondary infection groups. For fecal excretion data, fecal pellet samples and cecoliths were treated entirely separately.

In analyzing the GI tract at necropsy, comparisons were made only for the tract as a whole or within anatomical segment categories. Only a single uninfected rabbit was available to provide "control" values. Both absolute and relative concentrations of each nutrient for each segment for infected rabbits were compared with those of the control rabbit by t-test (mean vs. hypothesized value) with significance level at $P<.05$. Means for only two infected groups, primary infections at PIW 5 and 10-15 ($n=6$) and secondary infections at PIW 10-15 ($n=3$), were evaluated independently against the control value. (Primary and secondary infection groups at PIW 37-45 each consisted of only 2 rabbits and were considered too small to evaluate against a single control value; 1-way ANOVA of 5 infected groups was also considered unsuitable since 3 of the groups consisted of only 2 rabbits.)

[27]Microstat, version 4 (1984), Ecosoft, Inc., Zenith Data Systems Corp., St Joseph, MI 49085

RESULTS

Fecal excretion of N, P and Ca

The N, P, and Ca content of 204 fecal samples were analyzed, and the nutrient content of 174 from the primary infection group and 61 from the secondary infection group were contrasted with 30 samples from the uninfected group (Table 4.3). Nine rabbits contributed fecal samples while uninfected.

Primary group fecal samples included 100 from 12 patent infections (25.6% of samples collected during prepatency, 43.4% during patency, 20.4% during post-patency) and 12 (10.6%) from 2 nonpatent infections. Of the 14 primary infections, 11 were established by doses of 15,000 to 45,000 L3; 3 (7 fecal samples) were established by small doses (500, 1000, 2500 L3). Secondary group fecal samples included 18 from 2 patent infections (none prepatent, 11.5% collected during patency, 18.0% collected during postpatency) and 43 (70.5%) from 3 nonpatent infections. All secondary infections were generated by doses of 41,300 to 50,400 L3.

Anorexia was observed during PIW 1, 2, and 3 in 11 of the 14 primary infections. Of the 5 secondary infections, anorexia was observed during PIW 1 in 2 rabbits. Although clinically anorectic rabbits occasionally produced scanty fecal material of a diarrheic or potentially cecolithic consistency, only those fecal samples with normal appearance, consistency and odor were included in the present analysis.

Obeliscoides egg production began during PIW 2 to 7 (mean 23.1 d post-inoculation) in the 12 patent primary infections and PIW 4 and 5 (mean 30.5 d post-inoculation) in the 2 patent secondary infections. Patency ceased between PIW 12 to 27 among both primary and secondary infections. Egg production levels varied widely: the maximum observed was 541 epg, but in most cases was less than 20 epg (see also Nielsen, Chap. 1). Adult and immature or stunted Obeliscoides sp. were recovered at necropsy from all 9 of the 12 primary infections which terminated in necropsy, and in all 5 of the secondary infections which so terminated.

The fecal N content (Table 4.3) of infected rabbits collected during PIW 1 & 2 was significantly higher than that of both uninfected rabbits and of infected rabbits during other weeks

(except as below). This was true for both primary and secondary infections. Significantly elevated fecal N content continued during the following interval, PIW 3-5, but only for primary infections. The high mean N for these 3 groups, however, remained within the range of N values observed among the 30 uninfected control samples. No significant differences were found in the fecal P content of uninfected and various infected rabbit groups (Table 4.3).

During PIW 16-34, the fecal Ca content of the primary infected group significantly exceeded that of the control group or other infected groups (Table 4.3). The mean Ca content of this group also exceeded the largest Ca value observed among the 30 uninfected control samples. N content of these same fecal samples was not elevated, indicating these samples were not cecolithic or transitional fecal forms inadvertently identified as fecal pellets. Five rabbits contributed the 14 fecal samples analyzed during this interval; larval doses establishing these primary infections ranged from 15,000 to 36,000 L3. Five of the 14 samples were obtained during patency (1 to 33 epg), while 9 were obtained 2 to 14 weeks following the end of patency. Closer examination of the high-Ca group revealed that the largest Ca concentrations (44.9 to 52.0 mmol/100 g DM) were observed in 4 rabbits (without SQXN treatment) during the subinterval PIW 16-18.

For cecoliths, there were no significant differences in nutrient content (tested by one-way ANOVA) among the control (n=3), primary (n=14) and secondary (n=8) infected groups. When all 25 cecolith samples were contrasted with all 204 fecal pellet samples (Table 4.3), mean N content was nearly twice as large in the former group. Mean P content was somewhat larger in cecoliths than in fecal pellets. Mean Ca content was nearly the same for the two fecal types, but the cecolithic content of this mineral varied over a very large range.

GI segments at necropsy: DM, N, P, and Ca content

Tables 4.4, 4.5, 4.6 and 4.7 present the DM, N, P, and Ca values observed in each GI segment at necropsy. Also included are the size of the total GI tract pool (sum of all 8 segments), the antemortem food intake, and the balance between intake and GI pool for each nutrient. Necropsied rabbits are grouped on the basis of their Obeliscoides infection status: 8 had received primary inoculations of infective larvae (16,300 to 45,000 L3) between 5 and 45 weeks prior to necropsy, while 5 had received secondary inoculations (41,300 to 45,000 L3) between 10 and 45 weeks prior to necropsy.

TABLE 4.3. CONCENTRATION OF NUTRIENTS IN FECAL SAMPLES
from uninfected rabbits (controls) and from rabbits infected with Obeliscoides at various intervals (post-infection weeks, PIW). Data are expressed as mean ±SEM.

Treatment group	no. samples	N (g/100 g DM)		P (mmol/100 g DM)		Ca (mmol/100 g DM)	
(A) Regular fecal pellets							
Control	30	1.47	±0.03	32.10	±0.80	27.30	±0.72
Primary infections							
PIW 1&2	24	*1.76	±0.11	29.89	±1.23	31.74	±1.39
PIW 3-5	30	*1.74	±0.05	35.66	±0.75	31.61	±0.90
PIW 6-8	15	1.55	±0.06	34.78	±1.57	29.74	±1.28
PIW 9&10	8	1.68	±0.10	35.23	±1.65	28.66	±1.16
PIW 11-15	14	1.67	±0.05	35.24	±1.68	29.85	±1.10
PIW 16-34	14	1.65	±0.05	34.73	±1.42	*37.42	±2.72
PIW 35-45	7	1.54	±0.06	34.04	±1.83	31.01	±1.80
Secondary infections							
PIW 1&2	9	*1.73	±0.06	32.93	±2.34	30.63	±2.45
PIW 3-5	12	1.68	±0.06	36.67	±1.50	31.94	±1.14
PIW 6-8	15	1.55	±0.07	34.35	±1.99	28.94	±1.75
PIW 9&10	6	1.65	±0.08	36.97	±1.38	30.27	±1.36
PIW 11-15	4	1.53	±0.05	38.91	±3.28	32.06	±1.67
PIW 16-34	10	1.44	±0.06	31.90	±1.77	28.69	±1.90
PIW 35-45	6	1.52	±0.05	32.77	±1.20	28.78	±1.68
(B) Cecoliths (both uninfected and infected rabbits)							
	25	3.93	±0.11	41.76	±0.86	29.11	±1.23
	range:	(2.41-4.83)		(36.16-50.36)		(19.96-45.91)	

* Regular fecal samples with a nutrient concentration significantly different from the control group and from other infected groups at $P<.05$

TABLE 4.4 NUTRIENT CONTENT OF GI TRACT SEGMENTS AT NECROPSY: DRY MATTER (DM) CONTENT of specific segments and of the total tract, with pool=total dry grams in segment, % of total GI amount = grams DM as % total grams of GI DM, moisture (%) = weight of water as % total wet weight of ingesta in segment at necropsy, and grams/meter = grams DM per meter length of segment. Data are expressed as mean ±SEM for all groups except the single uninfected control rabbit; primary PIW 5 and PIW 10-15 are grouped for t-test.

Group	n	Pool size (g)	% total GI amount	moisture (%)	Concentration (g/m)
STOMACH					
Control	1	31.31 -	33.98 -	22.11 -	NA
Primary					
PIW 5	2	24.08±10.53	28.05 ±8.77	21.29 ±0.86	NA
PIW 10-15	4	31.28 ±9.68	34.84 ±4.13	23.15 ±0.78	NA
PIW 5-15	6			22.53 ±0.67	NA
PIW 37-45	2	36.69 ±7.56	43.08 ±4.25	23.98 ±0.52	NA
Secondary					
PIW 10-15	3	32.05 ±0.89	44.35 ±2.67	22.10 ±0.42	NA
PIW 37-45	2	32.27 ±9.94	34.81 ±7.09	23.30 ±1.24	NA
DUODENUM					
Control	1	0.29 -	0.31 -	6.69 -	0.90 -
Primary					
PIW 5	2	0.32 ±0.07	0.38 ±0.03	6.33 ±0.78	1.12 ±0.23
PIW 10-15	4	0.39 ±0.19	0.39 ±0.14	9.23 ±2.42	1.11 ±0.46
PIW 5-15	6			8.26 ±1.66	1.11 ±0.30
PIW 37-45	2	0.15 ±0.11	0.19 ±0.15	4.15 ±2.27	0.44 ±0.33
Secondary					
PIW 10-15	3	0.21 ±7.39	0.30 ±0.11	5.50 ±0.53	0.84 ±0.33
PIW 37-45	2	0.50 ±0.17	0.54 ±0.13	8.53 ±1.11	1.41 ±0.50

NA = not applicable

Table 4.4: DM, page 2

Group	n	Pool size (g)	% total GI amount	moisture (%)	Concentration (g/m)
ANTERIOR JEJUNUM					
control	1	2.86 -	3.10 -	10.63 -	2.86 -
primary					
PIW 5	2	2.00 ±0.17	2.48 ±0.22	10.13 ±1.60	2.55 ±0.33
PIW 10-15	4	1.48 ±0.65	2.55 ±0.44	13.62 ±0.29	2.66 ±0.60
PIW 5-15	6			*12.45 ±0.86	2.62 ±0.39
PIW 37-45	2	2.68 ±0.03	3.21 ±0.31	12.33 ±0.64	2.99 ±0.21
Secondary					
PIW 10-15	3	2.50 ±0.23	3.41 ±0.07	14.11 ±1.91	2.47 ±0.15
PIW 37-45	2	2.58 ±0.35	2.84 ±0.07	15.60 ±4.46	2.62 ±0.35
POSTERIOR JEJUNUM					
Control	1	4.33 -	4.70 -	19.96 -	4.33 -
Primary					
PIW 5	2	2.91 ±0.33	3.54 ±0.14	19.96 ±0.25	3.71 ±0.67
PIW 10-15	4	2.63 ±0.70	2.98 ±0.72	24.78 ±3.45	3.15 ±0.83
PIW 5-15	6			23.17 ±2.41	3.34 ±0.57
PIW 37-45	2	2.59 ±0.96	3.24 ±1.50	23.27 ±1.78	2.82 ±0.91
Secondary					
PIW 10-15	3	2.50 ±0.42	3.39 ±0.36	21.80 ±2.12	*2.46 ±0.35
PIW 37-45	2	4.28 ±1.63	4.58 ±1.28	23.28 ±1.25	4.39 ±1.59

* Significantly different from control value by means t-test at $P<.05$

Table 4.4: DM, page 3

Group	n	Pool size (g)	% total GI amount	moisture (%)	Concentration (g/m)
ILEUM					
control	1	1.07 -	1.17 -	23.35 -	2.90 -
primary					
PIW 5	2	0.87 ±0.20	1.05 ±0.09	23.55 ±1.40	2.58 ±0.85
PIW 10-15	4	0.87 ±0.25	1.02 ±0.31	31.27 ±6.19	2.39 ±0.63
PIW 5-15	6			28.70 ±4.25	2.46 ±0.46
PIW 37-45	2	0.90 ±0.06	1.08 ±0.05	23.67 ±0.93	2.50 ±0.03
secondary					
PIW 10-15	3	0.77 ±0.05	1.07 ±0.13	28.61 ±8.22	*2.11 ±0.14
PIW 37-45	2	0.72 ±0.05	0.80 ±0.03	28.82 ±2.87	2.01 ±0.21
CECUM					
Control	1	19.55 -	21.22 -	27.46 -	39.90 -
Primary					
PIW 5	2	22.45 ±1.17	27.72 ±2.58	26.80 ±0.23	44.97 ±3.24
PIW 10-15	4	22.11 ±3.88	26.28 ±1.59	27.45 ±0.58	45.66 ±6.93
PIW 5-15	6			27.24 ±0.39	45.43 ±4.47
PIW 37-45	2	17.45 ±2.65	20.61 ±0.89	27.99 ±0.65	41.87 (n=1)
Secondary					
PIW 10-15	3	17.40 ±1.27	24.27 ±2.95	27.23 ±1.29	37.82 ±2.61
PIW 37-45	2	20.72 ±3.22	22.75 ±1.02	29.45 ±1.15	46.08 ±9.61

* Significantly different from control value by means t-test at P<.05

Table 4.4: DM, page 4

Group	n	Pool size (g)	% total GI amount	moisture (%)	Concentration (g/m)
COLON					
control	1	10.91 -	11.84 -	28.11 -	33.05 -
primary					
PIW 5	2	12.12 ±0.63	14.97 ±1.40	28.06 ±0.09	34.18 ±2.25
PIW 10-15	4	10.16 ±1.25	12.30 ±0.75	28.26 ±0.80	29.30 ±2.92
PIW 5-15	6			28.19 ±0.51	30.93 ±2.19
PIW 37-45	2	12.47 ±1.00	15.12 ±2.85	27.81 ±1.60	42.87 ±7.04
secondary					
PIW 10-15	3	7.58 ±3.12	9.93 ±3.52	29.14 ±0.65	21.93 ±5.26
PIW 37-45	2	10.37 ±1.58	11.78 ±3.06	29.88 ±0.59	32.27 ±3.92
RECTUM					
Control	1	21.81 -	23.67 -	46.50 -	17.88 -
Primary					
PIW 5	2	17.36 ±1.17	21.81 ±4.58	49.32 ±0.26	15.52 ±2.14
PIW 10-15	4	15.73 ±2.49	19.64 ±3.47	45.72 ±2.04	13.36 ±1.68
PIW 5-15	6			46.92 ±1.50	*14.08 ±1.28
PIW 37-45	2	11.34 ±0.99	13.50 ±0.30	42.98 ±0.15	10.25 (n=1)
Secondary					
PIW 10-15	3	10.20 ±3.56	13.74 ±3.20	43.82 ±8.31	*10.58 ±2.71
PIW 37-45	2	19.21 ±3.68	21.92 ±6.50	50.11 ±0.64	34.00 ±22.23

* Significantly different from control value by means t-test at $P<.05$

Table 4.4: DM, page 5

GROUP	n	Pool size DM (g)	Change from DM intake (g)	Antemortem food intake: DM (g)
TOTAL, all segments				
control	1	92.13 -	4.46 -	87.67 -
primary				
PIW 5	2	82.12±16.78	13.23 ±9.79	68.89±18.79
PIW 10-15	4	85.42±31.72	22.77±21.84	62.63±26.07
PIW 5-15	6	84.32±10.51		64.71±17.23
PIW 37-45	2	84.25±13.05	31.02±26.33	53.23 ±9.39
secondary				
PIW 10-15	3	*72.99 ±9.04	26.95±14.97	*45.92±13.69
PIW 37-45	2	90.64±14.29	42.11 ±3.22	48.53 ±7.83

* Significantly different from control value by means t-test at P<.05

TABLE 4.5. NUTRIENT CONTENT OF GI TRACT SEGMENTS AT NECROPSY: NITROGEN (N) CONTENT of specific segments and of the total tract, with pool = total grams N in segment, % of total GI amount = grams as % total grams GI N, g N/100 g DM = grams of N per 100 grams DM, grams N/meter = grams N per meter length of segment. Data are expressed as mean ±SEM for all groups except the single uninfected control rabbit; primary PIW 5 and 10-15 are grouped for the t-test.

Group	n	Pool size (g)	%total GI N	Relative conc. (g N/100 g DM)	Absolute conc. (g N/m)
STOMACH					
control	1	0.57 -	21.98 -	1.81 --	NA
primary					
PIW 5	2	0.54 ±0.23	20.32 ±6.49	2.25 ±0.03	NA
PIW 10-15	4	0.77 ±0.26	26.77 ±3.48	2.45 ±0.15	NA
PIW 5-15	6			*2.38 ±0.10	NA
PIW 37-45	2	0.59 ±0.26	23.55 ±7.45	2.85 ±0.09	NA
secondary					
PIW 10-15	3	0.79 ±0.11	33.27 ±5.15	2.50 ±0.40	NA
PIW 37-45	2	0.83 ±0.22	30.45 ±5.71	2.62 ±0.12	NA
DUODENUM: content insufficient for N analysis					
ANTERIOR JEJUNUM					
Control	1	0.18 -	7.06 -	6.37 -	0.18 -
Primary					
PIW 5	2	0.14 ±0.01	5.55 ±0.73	6.96 ±0.46	0.18 ±0.02
PIW 10-15	4	0.14 ±0.03	5.10 ±0.97	6.34 ±0.34	0.17 ±0.04
PIW 5-15	6			6.55 ±0.28	0.17 ±0.02
PIW 37-45	2	0.17 ±0.09	7.09 ±1.35	6.22 ±0.23	0.19 ±0.02
Secondary					
PIW 10-15	3	0.15 ±0.02	6.09 ±0.56	5.89 ±0.42	0.15 ±0.01
PIW 37-45	2	0.16 ±0.01	5.92 ±0.26	6.23 ±0.59	0.17 ±0.01

* Significantly different from control value by means t-test at P<.05
NA Not applicable

Table 4.5: N, page 2

Group	n	Poll size (g)	% total GI N	Relative conc. (g N/100 g DM)	Absolute conc. (g N/m)
POSTERIOR JEJUNUM					
control	1	0.15 -	5.81 -	3.46 -	0.15 -
primary					
PIW 5	2	0.09 ±0.01	3.64 ±0.19	3.19 ±0.13	0.12 ±0.02
PIW 10-15	4	0.09 ±0.02	3.13 ±0.79	3.34 ±0.07	0.11 ±0.03
PIW 5-15	6			*3.29 ±0.07	*0.11 ±0.02**
PIW 37-45	2	0.08 ±0.03	2.97 ±0.87	2.83 ±0.16	0.08 ±0.03
secondary					
PIW 10-15	3	0.08 ±0.01	3.39 ±0.43	3.29 ±0.11	*0.08 ±0.01
PIW 37-45	2	0.17 ±0.05	6.09 ±1.47	4.02 ±0.28	0.17 ±0.05
ILEUM					
Control	1	0.04 -	1.35 -	3.24 -	0.09
Primary					
PIW 5	2	0.03 ±0.004	1.06 ±0.05	3.15 ±0.20	0.08 ±0.02
PIW 10-15	3	0.03 ±0.01	1.12 ±0.33	2.87 ±0.12	0.09 ±0.01
PIW 5-15	5			*2.98 ±0.12	0.08 ±0.01
PIW 37-45	2	0.02 ±0.003	0.95 ±0.25	2.44 ±0.13	0.06 0
Secondary					
PIW 10-15	2	0.02 ±0.001	0.88 ±0.01	2.89 ±0.38	*0.06 ±0.01
PIW 37-45	2	0.03 0	0.92 ±0.08	3.43 ±0.28	0.07 0

* Significantly different from control value by means t-test at P<.05

** Both relative (g N/100 g DM) and absolute (g N/meter) concentrations differ significantly from respective control values at p<.05

Table 4.5: N, page 3

Group	n	Poll size (g)	% total GI N	Relative conc. (g N/100 g DM)	Absolute conc. (g N/m)
CECUM					
control	1	0.86 -	33.29 -	4.39 -	1.75 -
primary					
PIW 5	2	1.01 ±0.05	39.80 ±2.82	4.48 ±0.01	2.02 ±0.15
PIW 10-15	4	1.00 ±0.23	35.81 ±0.86	4.40 ±0.36	2.06 ±0.43
PIW 5-15	6			4.43 ±0.23	2.05 ±0.28
PIW 37-45	2	0.82 ±0.07	33.16 ±5.92	4.56 ±0.52	1.69 (n=1)
secondary					
PIW 10-15	3	0.83 ±0.10	34.77 ±4.57	4.76 ±0.29	1.80 ±0.19
PIW 37-45	2	0.83 ±0.07	30.72 ±0.13	4.04 ±0.28	4.04 ±0.26
COLON					
Control	1	0.42 -	16.25 -	3.84 -	1.27 -
Primary					
PIW 5	2	0.49 ±0.04	19.15 ±0.70	4.01 ±0.15	1.37 ±0.14
PIW 10-15	4	0.42 ±0.07	15.60 ±0.97	4.06 ±0.26	1.21 ±0.18
PIW 5-15	6			4.04 ±0.17	1.26 ±0.12
PIW 37-45	2	0.55 ±0.10	22.63 ±0.97	4.38 ±0.46	1.91 ±0.51
Secondary					
PIW 10-15	3	0.31 ±0.12	12.75 ±4.70	*4.17 ±0.07	0.91 ±0.20
PIW 37-45	2	0.37 ±0.07	14.02 ±3.66	3.55 ±0.11	1.15 ±0.17

* Significantly different from control value by means t-test at P<.05

Table 4.5: N, page 4

Group	n	Poll size (g)	% total GI N	Relative conc. (g N/100 g DM)	Absolute conc. (g N/m)
RECTUM					
control	1	0.37 -	14.26 -	1.69 -	0.30 -
primary					
PIW 5	2	0.26 ±0.02	10.49 ±2.10	1.51 ±0.02	0.24 ±0.04
PIW 10-15	4	0.30 ±0.03	12.43 ±2.48	1.99 ±0.22	0.26 ±0.02
PIW 5-15	6			1.83 ±0.18	*0.25 ±0.01
PIW 37-45	2	0.23 ±0.01	9.66 ±1.78	2.02 ±0.09	0.22 (n=1)
secondary					
PIW 10-15	3	0.21 ±0.02	8.85 ±0.63	2.33 ±0.48	*0.23 ±0.01
PIW 37-45	2	0.28 ±0.06	10.78 ±3.17	1.47 ±0.04	0.21 ±0.04

GROUP	n	Pool size N (g)	Change from N intake (g)	Antemortem food intake: N (g)
TOTAL, all segments				
control	1	2.58 -	0.15 -	2.43 -
primary				
PIW 5	2	2.55 ±0.32	0.64 ±0.20	1.91 ±0.52
PIW 10-15	4	2.76 ±0.58	1.02 ±0.19	1.73 ±0.72
PIW 5-15	6	2.69 ±0.38		1.79 ±0.48
PIW 37-45	2	2.42 ±0.34	0.94 ±0.08	1.47 ±0.26
secondary				
PIW 10-15	3	2.40 ±0.07	1.13 ±0.33	*1.27 ±0.38
PIW 37-45	2	2.69 ±0.23	1.35 ±0.01	1.34 ±0.22

* Significantly different from control value by means t-test at P<.05

TABLE 4.6. NUTRIENT CONTENT OF GI TRACT SEGMENTS AT NECROPSY: PHOSPHORUS (P) CONTENT of specific segments and of the total tract, with pool = total millimoles (mmol) of P in segment, % total GI P = mmol in segment as % of total GI mmol P, mmol P/100 g DM = mmol P per 100 grams DM in segment, and mmol P/meter = mmol P per meter length of segment. Data are expressed as mean ±SEM for all groups except the single uninfected control rabbit; primary PIW 5 and 10-15 ara grouped for the t-test.

Group	n	Pool size (mmol)	%total GI P	Relative conc. (mmol P/100 g DM)	Absolute conc. (mmol P/m)
STOMACH					
control	1	2.48 -	9.50 -	7.91 -	NA
primary					
PIW 5	2	2.79 ±1.41	10.69 ±4.37	11.14 ±0.97	NA
PIW 10-15	4	4.46 ±1.70	17.15 ±3.63	13.72 ±1.09	NA
PIW 5-15	6			*12.86 ±0.91	NA
PIW 37-45	2	5.05 ±1.38	20.93 ±3.91	13.56 ±0.97	NA
secondary					
PIW 10-15	3	3.67 ±0.72	18.87 ±4.62	11.57 ±2.59	NA
PIW 37-45	2	5.41 ±0.65	18.13 ±2.66	17.84 ±3.47	NA
DUODENUM: content insufficient for P analysis					
ANTERIOR JEJUNUM					
Control	1	0.99 -	3.80 -	34.71 -	0.99 -
Primary					
PIW 5	2	0.72 ±0.01	2.92 ±0.32	35.76 ±1.70	0.91 ±0.08
PIW 10-15	4	0.76 ±0.18	3.06 ±0.60	33.86 ±0.59	0.91 ±0.21
PIW 5-15	6			34.49 ±0.70	0.91 ±0.14
PIW 37-45	2	0.89 ±0.08	3.77 ±0.01	33.34 ±2.51	1.00 ±0.14
Secondary					
PIW 10-15	3	0.83 ±0.13	4.11 ±0.33	32.77 ±2.44	0.81 ±0.10
PIW 37-45	2	0.86 ±0.07	2.87 ±0.32	33.42 ±1.78	0.87 ±0.07

NA Not applicaple
* Significantly different from control value by means t-test at P<.05

Table 4.6: P, page 2

Group	n	Poll size (mmol)	% total GI P	Relative conc. (mmol P/100 g DM)	Absolute conc. (mmol P/m)
POSTERIOR JEJUNUM					
control	1	1.41 -	5.41 -	32.61 -	1.41 -
primary					
PIW 5	2	1.02 ±0.14	4.08 ±0.07	34.79 ±0.89	1.30 ±0.27
PIW 10-15	4	0.88 ±0.25	3.48 ±0.95	31.80 ±1.98	1.05 ±0.30
PIW 5-15	6			32.80 ±1.42	1.13 ±0.21
PIW 37-45	2	0.89 ±0.32	3.89 ±1.68	34.55 ±0.65	0.97 ±0.30
secondary					
PIW 10-15	3	0.85 ±0.14	4.26 ±0.59	34.06 ±0.98	*0.84 ±0.12
PIW 37-45	2	1.66 ±0.49	5.58 ±1.79	40.12 ±3.80	1.68 ±0.49
ILEUM					
Control	1	0.33 -	1.25 -	30.35 -	0.88 -
Primary					
PIW 5	2	0.28 ±0.09	1.09 ±0.23	31.00 ±3.23	0.83 ±0.35
PIW 10-15	3	0.31 ±0.05	1.23 ±0.30	28.30 ±0.96	0.84 ±0.10
PIW 5-15	5			29.38 ±1.32	0.84 ±0.12
PIW 37-45	2	0.28 ±0.01	1.19 ±0.14	31.16 ±2.75	0.78 ±0.07
Secondary					
PIW 10-15	2	0.25 ±0.01	1.20 ±0.14	33.09 ±2.42	*0.65 ±0.02
PIW 37-45	2	0.28 ±0.01	0.95 ±0.06	39.23 ±2.10	0.79 ±0.05

* Significantly different from control value by means t-test at P<.05

Table 4.6: P, page 3

Group	n	Poll size (mmol)	% total GI P	Relative conc. (mmol P/100 g DM)	Absolute conc. (mmol P/m)
CECUM					
control	1	8.59 -	32.90 -	43.91 -	17.52 -
primary					
PIW 5	2	9.58 ±0.99	38.66 ±0.71	42.54 ±2.18	19.20 ±2.36
PIW 10-15	4	8.89 ±1.56	36.84 ±2.21	40.24 ±0.78	18.33 ±2.75
PIW 5-15	6			*41.00 ±0.89	18.62 ±1.85
PIW 37-45	2	7.29 ±0.44	30.82 ±0.98	42.38 ±3.96	16.09 (n=1)
secondary					
PIW 10-15	3	7.63 ±0.29	39.00 ±3.93	44.07 ±1.59	16.59 ±0.59
PIW 37-45	2	9.61 ±0.80	32.16 ±3.48	46.89 ±3.47	21.27 ±2.91
COLON					
Control	1	4.31 -	16.53 -	39.55 -	13.07 -
Primary					
PIW 5	2	4.85 ±0.49	19.58 ±0.43	39.88 ±1.94	13.68 ±1.56
PIW 10-15	4	3.93 ±0.41	16.66 ±0.91	38.98 ±1.35	11.34 ±0.95
PIW 5-15	6			39.28 ±1.00	12.12 ±0.88
PIW 37-45	2	4.74 ±0.28	20.28 ±3.04	38.10 ±0.81	16.28 ±2.34
Secondary					
PIW 10-15	3	2.96 ±1.20	14.22 ±4.75	39.50 ±0.19	8.58 ±1.99
PIW 37-45	2	4.88 ±0.94	16.23 ±2.72	46.82 ±1.94	15.19 ±2.47

* Significantly different from control value by means t-test at P<.05

Table 4.6: P, page 4

Group	n	Poll size (g)	% total GI P	Relative conc. (mmol P/100 g DM)	Absolute conc. (mmol P/m)
RECTUM					
control	1	7.99 -	30.62 -	36.64 -	6.55 -
primary					
PIW 5	2	5.62 ±0.11	23.01 ±3.20	32.45 ±1.62	5.00 ±0.44
PIW 10-15	4	4.94 ±0.75	21.68 ±3.89	31.64 ±1.40	4.19 ±0.49
PIW 5-15	6			*31.91 ±0.99	*4.46 ±0.37**
PIW 37-45	2	4.59 ±0.88	19.15 ±1.94	40.04 ±4.20	3.67 (n=1)
secondary					
PIW 10-15	3	3.73 ±0.58	18.59 ±1.67	38.74 ±2.08	*4.04 ±0.41
PIW 37-45	2	7.14 ±1.85	23.68 ±5.54	36.65 ±2.59	5.15 ±1.14

GROUP	n	Pool size P (mM)	Change from P intake (mM)	Antemortem food intake: P (mM)
TOTAL, all segments				
control	1	26.10 -	11.80 -	14.29 -
primary				
PIW 5	2	24.83 ±3.01	13.60 ±0.05	11.23 ±3.06
PIW 10-15	4	24.15 ±3.82	10.05 ±3.21	10.19 ±4.25
PIW 5-15	6	24.37 ±2.54		10.53 ±2.81
PIW 37-45	2	23.72 ±2.17	15.04 ±3.70	8.68 ±1.53
secondary				
PIW 10-15	3	*19.85 ±1.46	12.37 ±1.57	*7.49 ±2.23
PIW 37-45	2	29.95 ±0.78	22.04 ±2.06	7.91 ±1.28

* Significantly different from control value by means t-test at P<.05

** Both relative (millimoles P/100 g DM) and absolute (mmol P/meter) concentrations differ significantly from respective control values at p<.05

TABLE 4.7. NUTRIENT CONTENT OF GI TRACT SEGMENTS AT NECROPSY: CALCIUM (Ca) CONTENT of specific segments and of the total tract, with pool = total millimoles (mmol) of Ca in segment, % total GI Ca = mmol in segment as % of total GI mmol Ca, mmol Ca/100 g DM = mmol Ca per 100 grams DM in segment, and mmol Ca/meter = mmol Ca per meter length of segment. Data are expressed as mean ±SEM for all groups except the single uninfected control rabbit; primary PIW 5 and 10-15 are grouped for the t-test.

Group	n	Pool size (mmol)	%total GI Ca	Relative conc. (mmol Ca/ 100 g DM)	Absolute conc. (mmol Ca/m)
STOMACH					
control	1	2.89 -	16.74 -	9.23 -	NA
primary					
PIW 5	2	4.23 ±2.34	21.41 ±8.20	16.47 ±2.50	NA
PIW 10-15	4	5.59 ±2.93	25.59 ±8.07	14.85 ±3.50	NA
PIW 5-15	6			*15.39 ±2.33	NA
PIW 37-45	2	4.21 ±0.55	26.15 ±1.57	11.48 ±0.75	NA
secondary					
PIW 10-15	3	3.50 ±0.08 6	28.56 ±2.18	*10.93 ±0.22	NA
PIW 37-45	2	5.26 ±0.86	25.39 ±4.93	17.09 ±2.62	NA
DUODENUM: content insufficient for Ca analysis					
ANTERIOR JEJUNUM					
Control	1	0.27 -	1.53 -	9.23 -	0.27 -
Primary					
PIW 5	2	0.19 0	1.07 ±0.23	9.30 ±0.44	0.24 ±0.02
PIW 10-15	4	0.25 ±0.08	1.28 ±0.29	10.36 ±1.61	0.30 ±0.09
PIW 5-15	6			10.00 ±1.05	0.28 ±0.06
PIW 37-45	2	0.26 ±0.05	1.62 ±0.20	9.73 ±1.75	0.30 ±0.08
Secondary					
PIW 10-15	3	0.25 ±0.05	1.97 ±0.35	9.73 ±1.46	0.24 ±0.04
PIW 37-45	2	0.21 ±0.04	0.98 ±0.20	7.74 ±0.25	0.21 ±0.04

NA Not applicable
* Significantly different from control value by means t-test at P<.05

Table 4.7: Ca, page 2

Group	n	Poll size (mmol)	% total GI Ca	Relative conc. (mmol Ca/100 g DM)	Absolute conc. (mmol Ca/m)
POSTERIOR JEJUNUM					
control	1	0.09 -	5.19 -	20.71 -	0.90 -
primary					
PIW 5	2	0.76 ±0.09	4.23 ±0.43	25.95 ±0.13	0.96 ±0.18
PIW 10-15	4	0.58 ±0.18	3.12 ±0.93	20.49 ±2.18	0.70 ±0.21
PIW 5-15	6			22.31 ±1.79	0.79 ±0.15
PIW 37-45	2	0.61 ±0.19	3.90 ±1.45	24.08 ±1.62	0.67 ±0.18
secondary					
PIW 10-15	3	0.46 ±0.07	3.66 ±0.46	*18.34 ±0.62	*0.45 ±0.06**
PIW 37-45	2	0.93 ±0.31	4.53 ±1.63	22.21 ±1.25	0.95 ±0.31
ILEUM					
Control	1	0.23 -	1.33 -	21.46 -	0.62 -
Primary					
PIW 5	2	0.20 ±0.07	1.07 ±0.15	22.33 ±2.87	0.60 ±0.26
PIW 10-15	3	0.24 ±0.05	1.29 ±0.34	21.79 ±2.14	0.65 ±0.09
PIW 5-15	5			22.00 ±1.49	0.63 ±0.10
PIW 37-45	2	0.20 ±0.02	1.25 ±0.19	22.21 ±3.25	0.56 ±0.08
Secondary					
PIW 10-15	3	0.15 ±0.01	1.17 ±0.17	20.09 ±0.38	*0.40 ±0.01
PIW 37-45	2	0.18 ±0.01	0.85 ±0.08	24.33 ±0.38	0.49 ±0.04

* Significantly different from control value by means t-test at P<.05

** Both relative (mmol Ca/100 g DM) and absolute (mmol Ca/meter) concentrations differ significantly from respective control values at p<.05

Table 4.7: Ca, page 3

Group	n	Poll size (mmol)	% total GI Ca	Relative conc. (mmol Ca/100 g DM)	Absolute conc. (mmol Ca/m)
CECUM					
control	1	4.27 -	24.74 -	21.83 -	8.71 -
primary					
PIW 5	2	4.85 ±0.63	27.10 ±2.36	21.52 ±1.68	9.74 ±1.46
PIW 10-15	4	4.99 ±0.73	29.36 ±3.69	22.92 ±1.30	10.27 ±1.18
PIW 5-15	6			22.46 ±0.98	10.09 ±0.84
PIW 37-45	2	3.90 ±0.52	24.22 ±1.55	22.40 ±0.44	9.20 (n=1)
secondary					
PIW 10-15	3	3.63 ±0.40	30.00 ±5.26	20.96 ±2.13	7.92 ±0.96
PIW 37-45	2	5.21 ±0.27	25.05 ±2.10	25.51 ±2.68	11.50 ±1.22
COLON					
Control	1	2.37 -	13.72 -	21.71 -	7.18 -
Primary					
PIW 5	2	2.80 ±0.35	15.65 ±1.44	23.02 ±1.69	7.91 ±1.10
PIW 10-15	4	2.41 ±0.24	14.40 ±1.77	23.92 ±1.04	6.94 ±0.52
PIW 5-15	6			*23.62 ±0.81	7.26 ±0.48
PIW 37-45	2	2.84 ±0.31	17.95 ±3.17	22.21 ±0.13	9.78 ±1.87
Secondary					
PIW 10-15	3	1.59 ±0.65	12.29 ±4.19	21.25 ±0.63	4.64 ±1.07
PIW 37-45	2	3.05 ±0.70	14.52 ±2.88	29.01 ±2.31	9.45 ±1.88

* Significantly different from control value by means t-test at $P<.05$

Table 4.7: Ca, page 4

Group	n	Poll size (mmol)	% total GI Ca	Relative conc. (mmol Ca/100 g DM)	Absolute conc. (mmol Ca/m)
RECTUM					
control	1	6.34 -	36.74 -	29.07 -	5.20 -
primary					
PIW 5	2	5.23 ±0.44	29.50 ±3.91	28.57 ±2.75	4.63 ±0.07
PIW 10-15	4	4.08 ±0.61	25.23 ±4.86	26.23 ±1.34	3.47 ±0.41
PIW 5-15	6			27.01 ±1.21	*3.86 ±0.36
PIW 37-45	2	4.01 ±0.52	24.91 ±1.50	35.18 ±1.50	3.45 (n=1)
secondary					
PIW 10-15	3	2.85 ±0.49	22.71 ±2.48	29.40 ±1.36	*3.08 ±0.39
PIW 37-45	2	5.99 ±1.46	28.58 ±6.08	30.88 ±1.69	4.32 ±0.89

GROUP	n	Pool size Ca (mM)	Change from Ca intake (mM)	Antemortem food intake: Ca (mM)
TOTAL, all segments				
control	1	17.26 -	-7.44 -	24.70 -
primary				
PIW 5	2	18.25 ±3.91	-1.16 ±1.88	19.40 ±5.29
PIW 10-15	4	18.09 ±4.03	-2.09 ±1.50	17.64 ±7.34
PIW 5-15	6	18.15 ±2.74	*-1.95 ±1.07	18.23 ±4.86
PIW 37-45	2	16.01 ±1.12	1.02 ±3.77	14.99 ±2.65
secondary				
PIW 10-15	3	*12.39 ±0.85	*-0.54 ±3.15	*12.94 ±3.86
PIW 37-45	2	20.83 ±0.68	7.65 ±3.39	13.67 ±2.20

* Significantly different from control value by means t-test at P<.05

Widely varying numbers of Obeliscoides sp. were recovered from the stomachs at the time of necropsy, with parasite numbers decreasing with time post-inoculation and in secondary infections. Primary infections necropsied during PIW 5 (n=2) averaged 30,900 Obeliscoides, those during PIW 10-15 (n=4) averaged 2300 Obeliscoides, and those during PIW 37-45 (n=2) averaged 700 Obeliscoides. Secondary infections necropsied during PIW 10-15 averaged 150 Obeliscoides, while those necropsied during PIW 37-45 averaged 45 Obeliscoides.

Although all rabbits were fed their normal daily ration at the habitual feeding time between 3.5 and 4.0 hours prior to anaesthesia and subsequent euthanasia, the actual amount of chow intake (measured as g DM of chow absent from the feed hopper) varied considerably among individuals, ranging from no intake to ingestion of the entire ration (Table 4.4). The single uninfected control rabbit happened to have the second-largest antemortem food intake, while the 3 rabbits experiencing secondary infections at PIW 10-15 had tended, as usual, to delay eating. These intake variations had two consistent effects on GI tract segment analysis. (1) The total GI pool size of each nutrient were significantly less for the infected groups when compared to the control. In addition, for all 13 infected rabbits the mean total GI pool size of DM (82.7 g, SE= ±5.24) was significantly (P=.048) smaller than that of the control rabbit (92.2 g). (2) The absolute amounts of all nutrients in the posterior jejunum and ileum were significantly less from the secondary PIW 10-15 group when compared to the control group.

Because of these differences in the intake and resultant GI nutrient content of individuals, and in the absence of an insoluble reference marker within the GI lumen, only segmental nutrient concentrations that significantly differed from the control value both in absolute amount (amount per meter) and relative to the movement of DM (amount/100 g DM) or water (for DM analysis only) were considered of importance.

There were no significant relationships between infection status and DM content of any GI segment proximal to the rectum if both relative DM (% moisture) and absolute DM (g DM/meter) were considered (Table 4.4). Gastric DM content ranged from 13.5 to 59.8 g, with these extremes occurring in the individuals with lowest and highest intakes, respectively. Gastric DM content, the inverse of gastric fluid content, was relatively constant for all 14 rabbits (Table 4.4). Ten of the 11 rabbits which had intakes larger than 40 g had lost between one half and two-thirds of this DM amount from the stomach by the time of necropsy. In agreement, relative gastric DM content (as %

of all DM in the GI tract) was not directly related to recent feed intake: it ranged from 19.3% for the single rabbit with no intake prior to necropsy to 48.5% in the rabbit with the second-lowest intake.

For the nutrients N, Ca and P, significant differences were observed simultaneously in both concentration and amount per unit length in 3 cases (noted in Tables 4.5, 4.6, and 4.7 by double asterisks) for Obeliscoides-infected rabbits. (1) In primary-group rabbits during PIW 5-15, the N content of the posterior jejunum (Table 4.5) was significantly (P=.025 and .049) reduced, and simultaneously (2) the P content of the rectum (Table 4.6) was significantly (P=.003 and .001) lower than observed in the uninfected rabbit. (3) In secondary-group PIW 10-15 rabbits, the Ca content of the posterior jejunum (Table 4.7) was significantly (P= .031 and .010) reduced.

Other significant differences in either relative or absolute nutrient amounts were occasionally encountered when comparing specific GI segments of infected and control rabbits, as noted (by a single asterisk) in Table 4.5, 4.6 and 4.7. Of particular interest was the low N content of the posterior jejunum of both primary- and secondary-infected rabbits during PIW 5 and 10-15.

While the total GI pools of DM, N and P exceeded the immediate antemortem intake of these nutrients (Tables 4.4, 4.5 and 4.6), the amount of Ca in the entire tract was often smaller than its intake (Table 4.7). This negative Ca balance was present in 6 of the 14 rabbits, including the control rabbit. All 13 infected rabbits together had a mean Ca balance of +0.3 mmol (SE= ±1.36) that was significantly larger than that of the control rabbit (-7.4 mmol). Both primary and secondary infected groups had Ca balances that were less negative than that of the control rabbit (Table 6). This suggests that infected rabbits were absorbing less of this mineral over the tract as a whole, despite the indication of significantly enhanced Ca absorption noted in the posterior jejunum of the latter group.

The single uninfected control rabbit retained an unusually large amount of material within its rectum compared to the 13 infected rabbits. The presence of gastric trichostrongylids may have enhanced rectal emptying, but this cannot be determined with only a single uninfected control. The result, however, was that rectal values were consistently higher in the uninfected rabbit for all nutrients. This was only in absolute and not in relative terms, so that consistently high percentages of total GI nutrients occupied the rectum of the control rabbit in comparison to the other 13 rabbits.

The control rectum held amounts of DM (23.7%) and N (14.3%) that were significantly (P<.01) large compared to the means (DM: 17.9%, SE= ±1.73; N: 10.6% ±0.93) for all 13 infected rabbits. In the control rabbit, amounts of P (30.6%) and Ca (36.7%) were considerably (P<.0001) larger than for the 13 infected rabbits (21.1% ±1.47; Ca: 25.8% ±1.80). On a relative basis (amount per 100 g DM), however, rectal nutrient concentrations differed only in the single case cited above (P, primary 5-15).

Determination of GI passage rate parameters

Fecal Ce-141 concentrations were used to determine TT, TMRT and GITT for passage of the fiber component of the ingesta. These values were grouped into uninfected and various infected groups as listed in Table 4.8. Similarly, fecal concentrations of Cr-51-EDTA determined passage of the liquid component of the ingesta (including TMRT with and without oral SQXN treatment) are reported in Table 4.9.

Passage rate parameters were measured from 9 uninfected control rabbits on 11 occasions in 4 RI trials (Tables 4.8 and 4.9). No significant differences (P>.05) were found between rabbits treated with SQXN and those not treated, for any of the fiber passage parameters, or for TT and GITT of the liquid component. However, liquid TMRT was significantly (P=.02) prolonged on the 4 occasions when uninfected rabbits were treated with SQXN. Therefore, liquid TMRT comparisons for Obeliscoides-infected rabbits were done separately as SQXN-treated and -untreated categories.

TT for fiber (4.7 h) and liquid (5.1 h) components of the ingesta of uninfected rabbits were not significantly different. TMRT for the liquid component both without (53.0 h) and with SQXN (70.8 h) were both significantly (P<.001) longer than for the fiber component either without (18.9 h) and with SQXN (21.6 h). Similarly, GITT of liquid (57.0 h) was significantly prolonged (P<.001) compared to that of fiber (16.6 h). (Significance was determined by t-test of means with pooled variance.)

Among the 9 uninfected rabbits with passage rate parameters determined in the 4 10-day RI trials, fiber component marker concentration declined below detectability between 60 and 130 hours, while liquid component marker (Cr-51-EDTA) concentration remained detectable in all cases when the trials terminated at 240 h. Liquid marker concentrations were inevitably higher in cecoliths

TABLE 4.8. GASTROINTESTINAL FIBER PASSAGE RATE (h)#
in rabbits with primary and secondary Obeliscoides infections, at intervals post-infection (post-infection week, PIW). Data are expressed as mean ±SEM.

Treatment group	no. rabbits	Transit time		Total mean retention time		GI turnover time	
control	11	4.7	±0.4	19.9	±2.0	16.6	±0.9
primary infections							
PIW 2-3	8	6.5	±1.3	24.5	±3.9	16.7	±2.0
PIW 8-9	3	6.8	±1.9	20.0	±4.3	16.9	±0.9
PIW 16-36	8	4.6	±0.6	23.1	±1.8	18.7	±1.0
secondary infections							
PIW 2-3	3	4.0	±0.6	16.1	±3.1	15.1	±1.8
PIW 8-9	3	6.2	±0.1	23.5	±1.7	17.3	±2.2
PIW 16-26	4	5.4	±0.6	31.8*	±2.8	26.3*	±3.2

Determined by fecal excretions of Ce-141
* Significantly different from control group and other treatment groups in the same column at $P<.05$

TABLE 4.9. GASTROINTESTINAL LIQUID PASSAGE RATE (h)#
in rabbits with primary and secondary Obeliscoides infections, at intervals post-infection (post-infection week, PIW). TMRT subdivided according to concurrent oral SQXN treatment (see text). Data are expressed as mean ±SEM.

Treatment group	no. rabbits	Transit time		Total meam retention time no SQXN		Total meam retention time with SQXn		GI turnover time	
control	11	5.1	±0.4	53.0	±4.8	70.8	±5.5	57.0	±5.7
primary infections									
PIW 2-3	8	7.4	±1.3	50.6	±11.6	60.8	±7.7	53.5	±8.7
PIW 8-9	3	6.8	±1.9	xx		64.3	±10.6	57.5	±10.0
PIW 16-36	8	5.1	±0.7	62.9	±5.0	85.6	±7.2	61.4	±5.1
secondary infections									
PIW 2-3	3	4.0	±0.6	xx		57.8	±9.7	59.5	±10.4
PIW 8-9	3	6.9	±0.7	xx		84.3	±5.7	79.4	±8.6
PIW 16-26	4	5.5	±0.5	65.1	±5.8	xx		60.2	±6.7

\# Determined by fecal excretions of Cr-51-EDTA
xx values not available

than in regular fecal pellets excreted near the same time. For regular fecal samples, the terminal part of marker concentration vs. time curve frequently demonstrated a diurnal cyclicity based on the increase and decrease of concentrations of both component markers (Figure 4.1). Time intervals on the marker excretion curve without samples represented those periods without regular fecal pellet production, i.e. periods of cecotrophic re-ingestion.

The characteristic odor of VFA's was easily detected on the breath of rabbits engaged in cecotrophy, and the timing of the cecotrophic- and ration-ingestion periods seemed to be determined in large part by individual preference. As far as could be determined, the administration of oral SQXN did not affect daily timing of feeding and cecotrophy characteristic of particular individuals. Some individuals tended to "drop" cecoliths regularly at the beginning or end of cecotrophic periods, while others rarely dropped cecoliths. Occasionally, a few semi-firm but unusually large fecal pellets with a very mild VFA odor, an apparent "transitional" form of feces, were observed at the end of the cecotrophic period.

Infected rabbits for which GI passage parameters were determined included 13 rabbits measured on 19 occasions during 5 RI trials. Three primary infections evaluated in RI-1 were established by doses of 500, 1000 and 2500 L3; 10 primary infections evaluated in the remaining 4 trials were established by doses of 15,000 to 45,000 L3. Five secondary infections evaluated in these 4 RI trials were established by doses of 41,300 to 50,400 L3. The 13 primary infections were analyzed as 19 studies, divided into 3 post-inoculation intervals extending to PIW 36. The 5 secondary infections were analyzed as 10 studies, divided into 3 PIW intervals extending to PIW 26. SQXN treatment coincided with 10 of the 19 primary infection determinations and 6 of the 10 secondary infection determinations.

As in uninfected rabbits, liquid marker concentrations in infected rabbits were inevitably higher in cecoliths than in regular fecal pellets excreted near the same time. As long as rabbits were not anorectic, daily timing of feeding and cecotrophy characteristic of particular individuals was unchanged by infection. Two rabbits, both with primary infections during PIW 2 and 3, were moderately anorectic during the initial part of an RI trial. In both cases, values for GI passage parameters were available from these same individuals prior to their infection (Table 4.10). Anorexia prolonged all parameters for both fiber and liquid components in both rabbits, except liquid GITT in one individual. The terminal excretion component of fiber marker concentration for one of these

rabbits had a bipartite structure reflecting a change in slope (k) near 70 hours post-dosing, coincident with appetite recovery by this individual (Figure 4.2).

Fiber passage rate parameters of 4 groups (1 uninfected plus 3 infected, at different PIW intervals) were compared separately for primary and secondary infections by one-way ANOVA (Table 4.8). TT for fiber was prolonged in some of the infected groups, but the differences were not significant. Both TMRT and GITT of fiber, however, were significantly ($P<.01$) prolonged in a single infected group: secondary infections during PIW 16-26. This group included 4 determinations made on 2 rabbits in 2 RI trials, without SQXN treatment. Figure 4.3 provides an example of one of these determinations for which fiber TMRT=38.3 h and GITT=32.9 h.

TT and GITT of the liquid phase for the 4 groups (1 uninfected plus 3 infected) were similarly compared separately for primary and secondary infections (Table 4.9). No significant differences were noted among these groups. TMRT's were compared to relevant SQXN-treated or untreated control groups (a t-test of means with pooled estimate of variance was used to compare untreated control TMRT with untreated secondary infections during PIW 16-26, since only 2 groups were involved). Again, no significant differences were noted among these groups. Although within-group variations of liquid TMRT and GITT (Table 4.8) appeared to be large compared to variations within fiber TMRT and GITT (Table 4.9), relative variability (maximum observed SE/TMRT, maximum observed SE/GITT) were nearly identical for liquids (22.9%) and fiber (21.4%) just as CV's were similar (Table 4.2).

DISCUSSION

Nutrient absorption and fecal excretion in uninfected rabbits

Outside of the anorectic period, daily timing of ration intake, fecal pellet production, and cecotrophy was unchanged by gastric Obeliscoides infection. On the day of necropsy, none of the segment-analysis rabbits had been anorectic within the previous 4 to 51 weeks, yet considerable variations were observed in antemortem DM intake. These differences reflected individual preferences in the timing of daily activities (eating, grooming, sleeping and cecotrophy) that had been observed during each animal's sojourn in the colony prior to trichostrongylid infection.

TABLE 4.10. GASTROINTESTINAL PASSAGE RATES (h) FOR 2 RABBITS measured:

(a) prior to infection, with normal ration intake, and

(b) following Obeliscoides infection, with moderately reduced intake.#

Concurrent SQXN treatment is as noted.

Rabbit	Fiber passage rate			Liquid passage rate			
Treatment:	TT	TMRT	GITT	TT	TMRT		GITT
					no SQXN	with SQXN	
Rabbit 1127							
(a) Uninfected	4.2	19.2	17.6	4.2	54.9	xx	52.9
(b) Infected PIW 2-3	5.7	20.8	19.8	5.7	xx	73.6	76.3
Rabbit 0990							
(a) Uninfected	5.3	30.4	18.6	5.3	xx	68.2	71.5
(b) Infected PIW 2-3	14.8	47.9	20.0	14.8	xx	82.8	68.0

\# In both individuals the interval between the set of RI passage measurements was 5 weeks

xx Values not available

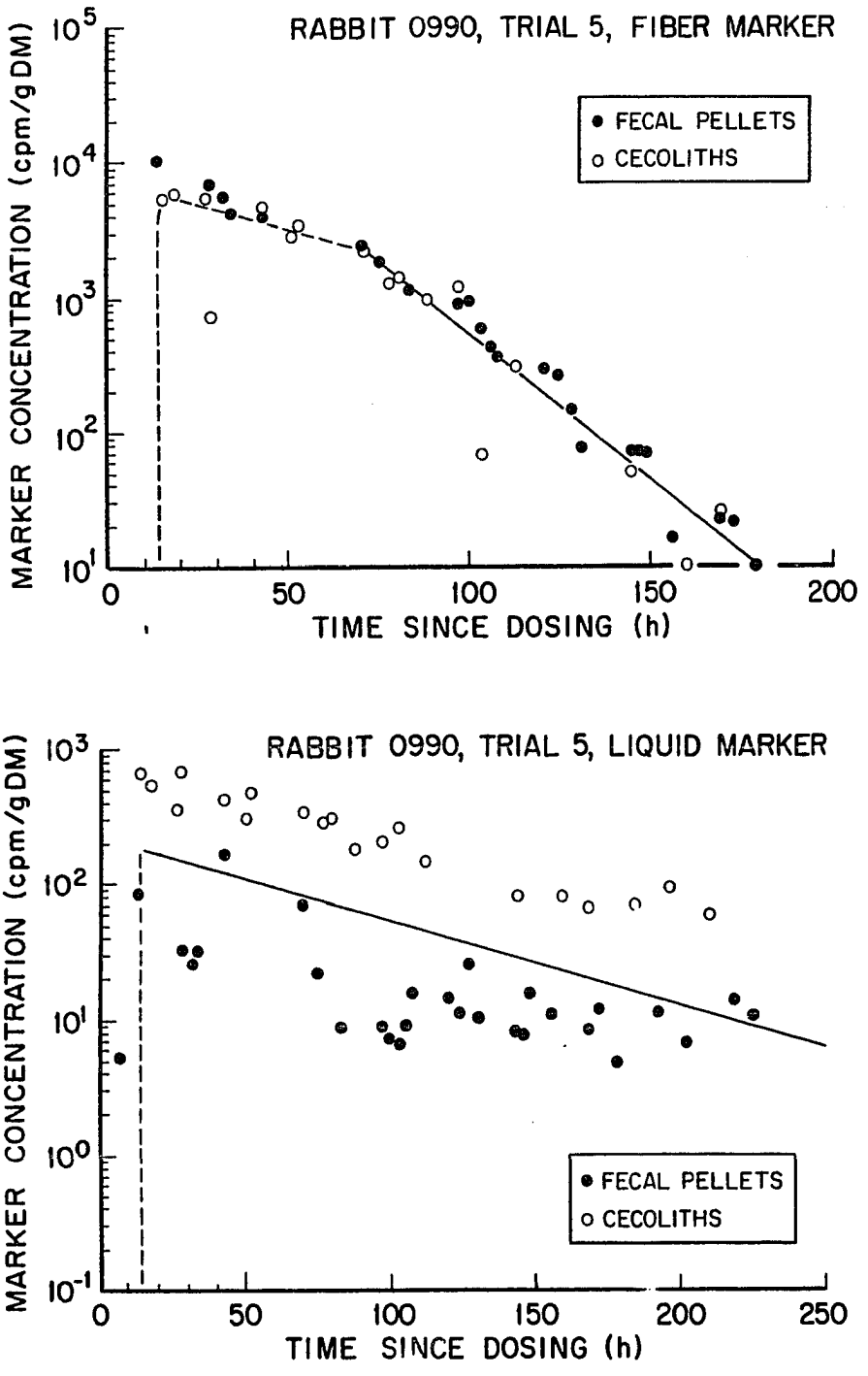

Figure 4.2 Anorectic infected rabbit: Concentration of radioisotopic marker in feces (cpm/g DM) vs. time since marker dosing (h). Top: Concentration of the fiber-component marker, Ce-141. Note the change in slope of the terminal excretion component coinciding with appetite recovery at 70 h. In this case, TT= 14.8, TMRT= 47.9 and GITT (after 70 h)= 20.0 h. Bottom: Concentration of the liquid-component marker, Cr-51-EDTA; TT= 14.8, TMRT= 82.8 and GITT= 68.0 h.

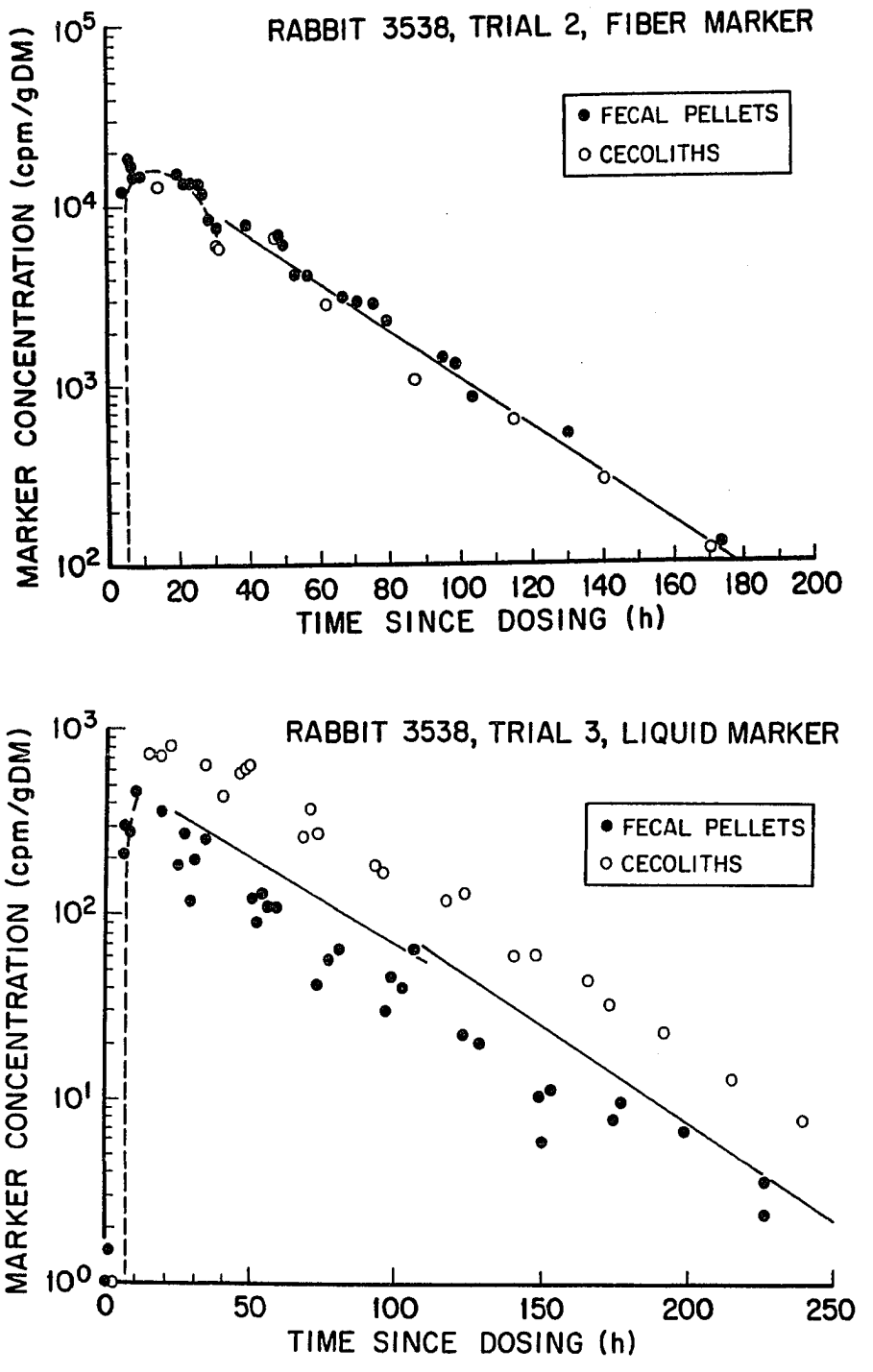

Figure 4.3 Prolonged fiber passage in a secondary-infection rabbit, PIW 16-26: Concentration of roadioisotopic marker in feces (cpm/g DM) vs. time since marker dosing (h). Top: Concentration of the fiber-component marker, Ce-141; TT= 5.0, TMRT= 38.3 and GITT= 32.9 h. Bottom: Concentration of the liquid-component marker, Cr-51-EDTA; TT= 7.0, TMRT= 51.6 and GITT= 41.0 h.

DM within the GI lumen varied according to antemortem intake and defecation, as had been previously reported (Carmichael et al, 1945). Segment-specific analysis of nutrient concentrations reported previously for rabbits (Huang et al, 1954) was done on a percentage basis that cannot be compared directly with the present study. Nutrient concentrations (relative to DM) within different segments of the GI tract were examined only for a single uninfected rabbit. Similar studies of the nutrient content of GI tract segments in ruminants utilized nonabsorbable reference markers to evaluate nutrient losses and gains along the tract (White et al, 1984; Staaland et al, 1986). Interpretation of both the present observations and a previous study of the rat (Shiga and Morino, 1986) are limited by the lack of such a marker. To improve the utility of the present study, changes in segmental nutrient concentrations were considered of importance only when both absolute and relative amounts were significantly different from those of the control.

N, Ca and P content per unit jejunum and ileum length were relatively uniform for all rabbits. A steady decline in absolute amounts of both N and P along the small intestine is consistent with this organ being a primary absorption site as observed in other species (Strombeck et al, 1979; Bivin et al, 1979; Scott and McLean, 1981; Staaland et al, 1984; White et al, 1984).

Estimations of absolute rates of absorption are complicated by the unknown amounts of endogenous nutrients added during digestion. Segmental analysis of N distribution in rats suggests 70% to 83% of the N is of endogenous origin within the distal small intestine (Partridge et al, 1985; Krawielitzki et al; 1987).

Segmental N content observed in the present study was similar to previous observations of the stomach and lower GI tract for rabbits on a minimally positive N balance diet (Hoover and Heitmann, 1975), while rectal and fecal N contents (1.7 and 1.5 g/100 g DM) were somewhat higher than reported for feces (1.314 g/100 g DM). In rabbits, relatively large amounts of urea-N enter the cecum from both the small intestine and from blood (Knutson, 1977; Hornicke and Bjornhag, 1980). While there is some evidence of amino acid diffusion from the cecum and colon, the large disappearance of N from the lower colon results primarily from the absorption of N as ammonia (Hoover and Heitmann, 1975; Hornicke and Bjornhag, 1980; Stevens et al, 1980). Reingestion of N-rich cecoliths means the small intestine remains the primary absorption site for amino acids (Hornicke and Bjornhag, 1980; Cheeke, 1987).

Fecal N, Ca and P concentrations in 30 regular fecal pellet samples from uninfected rabbits (listed first) were comparable with previous observations (range listed second): N, 1.47 vs. 1.12 to 2.99 g/100 g DM; P, 0.99 vs. 0.24 to 1.3 g/100 g DM; and Ca, 1.09 vs. 0.4 to 1.4 g/100 g DM (Kandatsu et al, 1959; Hornicke and Bjornhag, 1980; Pehrson, 1983; Cheeke, 1987; Fekete, 1989). In the present study, cecoliths were collected casually from both uninfected and infected rabbits, but their nutrient content also was comparable to that previously reported (often for collared rabbits), with more N and slightly more P than in regular fecal pellets: N, 3.93 vs. 3.84 to 5.98 g/100 g DM; P, 1.29 vs. 0.9 to 2.2 g/100 g DM; and Ca, 1.17 vs. 0.5 to 1.3 g/100 g DM (Kandatsu et al, 1959; Hornicke and Bjornhag, 1980; Pehrson, 1983; Cheeke, 1987; Fekete, 1989).

Nutrient absorption and excretion in infected rabbits

Increased fecal concentrations of N relative to DM occurred in the early weeks of both primary and secondary gastric Obeliscoides infections. The timing of this enhanced fecal N loss coincided with the prepatent and early patent periods of Obeliscoides infection, and included the weeks in which clinical anorexia was observed in many of the rabbits. In primary infections, significant increases in fecal N concentration continued to PIW 5. These results are consistent with previous observations (ending at PIW 3) of Obl. cuniculi in rabbits (Pace and Frandsen, 1982). Increased fecal N excretion in the prepatent and early patent periods is one of the most common lesions associated with reduced N digestibility in ruminant Ostertagia infections (Steel, 1974; Sykes and Coop, 1977; Randall and Gibbs, 1981; Parkins et al, 1982a, b; Verstegen et al, 1988; Fox, Gerrelli, Pitt, Jacobs, Gill and Gale, 1989).

For 6 rabbits with primary infections necropsied between PIW 5 and 15, N concentrations in the posterior jejunum were significantly reduced despite relatively high gastric N content, which is consistent with enhanced N absorption by the small intestine. These observations suggest that in primary Obeliscoides infections, the first 5 post-inoculation weeks are characterized by increased fecal N loss, and the following 10 weeks constitute a period of enhanced N absorption in the jejunum. Five weeks was also the length of a period of reduced N retention observed in protein-supplemented cattle harboring inhibited Ost. ostertagi larvae (Parkins et al, 1982b).

Compensatory enhancement of N absorption by the distal small intestine has long been advanced as a primary mechanism for limiting N losses associated with abomasal trichostrongylids

as compared to the larger losses induced by intestinal forms (Steel, 1974; Sykes, 1977; Steel and Symons, 1982; Symons, 1989). During the initial part of the period of enhanced N absorption in the present study (during PIW 6 and 7), serum gastrin concentrations increased significantly (Nielsen, Chap 3). Since gastrin has a trophic effect upon the gastrointestinal mucosa (Yau, 1982; Snider et al, 1985), this hormone may have mediated histological changes within the small intestine that resulted in the observed absorption increases. Gastric trichostrongylid infections in rabbits are associated with a marked local hyperplasia (Russell et al, 1970) ascribed at least in part to the trophic effects of gastrin (Snider et al, 1985; Nielsen, Chap. 1).

The capacity for compensatory intestinal absorption has previously been demonstrated in laboratory rodents and lagomorphs, and may be similar to mechanisms increasing digestive enzyme activity in the distal jejunum and ileum upon direct surgical excision of the proximal small intestine of rats (Chaves et al, 1987). Small doses of various infective nematode larvae have been found to stimulate the absorption of certain nutrients within the first several weeks post-infection, while larger doses diminish absorption (Symons, 1978; Sudkhdeo and Mettrick, 1984; Hsu et al, 1985). An "adaptive hyperplastic region" of the small intestine, distal to the main infection site, was histologically characterized in rats (Symons, 1978) and was also reported in rabbits dosed with 20,000 L3 intestinal trichostrongylids of ruminant origin, but was absent at low (500 L3) and high (30,000 L3) doses (Hoste et al, 1988; Hoste and Mallet, 1990).

Enhanced fecal N loss is often evaluated in terms of N, or protein, digestibility. Diminished protein digestibility has accompanied reduced intake during the early post-inoculation period in both rabbit Obeliscoides (Pace and Frandsen, 1982) and ruminant Ostertagia infections (Sykes and Coop, 1977; Randall and Gibbs, 1981; Parkins et al, 1982a; Fox, Gerrelli, Pitt, Jacobs, Gill and Gale, 1989). However, even cattle with normal intakes and excess protein in their ration have manifested chronic reductions in N retention and lower productivity (Parkins et al, 1982b).

In secondary Obeliscoides infections, clinical anorexia was briefer and indications of enhanced intestinal N absorption during PIW 10 to 15 were not significant. These findings suggest that immunological factors may mitigate functional causes of increased fecal N loss or other physiological alterations. Evidence for reduced growth and production associated with diminished nutrient digestibility has been equivocal for secondary Ostertagia infections in ruminants (Brunsdon, 1986; Jeffcoate et al, 1988; Verstegen et al, 1988; Ploeger, Kloosterman and Borgsteede, 1990). The

importance of the immune status of the host in determining the size and pattern of N loss induced by trichostrongylid infections requires further investigation (Sykes, 1987).

Rectal P content of rabbits with primary Obeliscoides infection was significantly reduced during PIW 5-15 (Table 4.6), coincident with the enhanced N absorption (Table 4.5). However, no significant changes were noted in fecal P excretion during this or any other interval. The relatively large rectal fill of the control animal may be a factor in this finding; exploration of the link between enhanced jejunal N and rectal P absorption must await a larger-scale study. The presence of P-rich cecoliths within the rectum also could influence the P content of this segment.

Fecal Ca concentrations significantly increased in the primary infection group during PIW 16-34, particularly during PIW 16-18. Rabbits were clinically normal at this time and infections were in the late-patent or post-patent stages. Similar observations have been reported in Ost. circumcincta infections in sheep, where fecal Ca excretion increased during PIW 4-6 (Wilson and Field, 1983). No rabbits were necropsied during this period to allow a segmental analysis of the site of the Ca change. Increased serum gastrin has been reported to enhance fecal Ca excretion in lambs (Barlet, 1973), but this regulatory mechanism seems unlikely in the present study because serum gastrin concentrations were normal during this interval (Nielsen, Chap. 3). In addition, serum Ca and P levels, reported elsewhere (Nielsen, Chap. 2), remained unchanged during both primary and secondary Obeliscoides infections.

A significant reduction in the amount of Ca in the posterior jejunum was observed during PIW 10-15 for the secondary infection group, suggesting that Ca absorption was transiently enhanced. This finding contrasts with the timing of increased fecal Ca excretion observed in the primary infection group during PIW 16-34, and with the observation that at necropsy all 13 infected rabbits were absorbing less Ca over the GI tract as a whole than the uninfected rabbit (Table 4.7). Unlike N and P, the absolute concentrations of Ca did not decrease steadily down the length of the small intestine, and relatively wide variations were seen in the Ca content of both regular fecal samples and cecoliths. These observations suggest that substantial fluctuations of Ca within the GI lumen over time may be physiologically normal for rabbits. Although intestinal Ca absorption has been considered a strictly regulated process in most mammals (Kenny, 1981), it is relatively unregulated in the rabbit (Cheeke, 1987), and wide variations in Ca content of the small intestine in reindeer (White et al, 1984; Staaland et al, 1986) and red deer (White et al, 1991) suggest this may be true

of other species as well.

Since Ca absorption is influenced by its solubility and the presence of potential co-precipitants in the lumen (Kenny, 1981; White et al, 1984; Shiga et al, 1987), Obeliscoides-induced pH changes in the proximal small intestine may have reduced absorption. The transient increase in Ca absorption observed in the distal small intestine in the present study may then have been compensatory. In rats, increased alkalinity of the duodenum and proximal jejunum substantially reduced local Ca absorption even when solubility was maintained, but distal absorption of the mineral increased (Waldron-Edward et al, 1966). The distal jejunum and ileum of rats have a substantial capacity for compensatory Ca absorption against concentration gradients (Kimberg et al, 1961).

Gastric pH shifts toward alkalinity are observed in Ostertagia-infected ruminants and are often associated with increased in serum gastrin concentrations (Titchen, 1982; Holmes, 1985; Symons, 1989). Gastrin also may indirectly influence Ca absorption, quite apart from its pH effects (Barlet, 1973; Yau, 1982). In the present study, serum gastrin concentrations significantly increased during PIW 6 and 7 in the primary infection group (Nielsen, Chap. 3). However, such changes were not associated with fecal Ca excretion, nor were there significant alterations in jejunal Ca among rabbits necropsied during PIW 5 or 10-15.

GI passage rate parameters in uninfected rabbits

GI passage rate parameters have traditionally been difficult to quantify for rabbits. Fiber passage is generally rapid among small herbivores, even in those with hindgut fermentation systems, possibly in association with limitations imposed by small body size (Leng et al, 1977; Hornicke and Bjornhag, 1980; Uden and Van Soest, 1982; Uden et al, 1982; Bjornhag, 1987; Sakaguchi et al, 1987; White et al, 1987; Sakaguchi and Hume, 1990). TT for the fiber component of rabbit ingesta has been determined at 3 to 9 h (Pickard and Stevens, 1972; Jilge, 1974; Sakaguchi et al, 1987; Sakaguchi and Hume, 1990), which is in agreement with 4.7 h for uninfected rabbits in the present study. While 19.9 h was the TMRT measured for fiber here, previous observations of TMRT vary widely between 6.21 and 90.5 (Hoover and Heitman, 1972; Sakaguchi et al, 1987; Sakaguchi and Hume, 1990). This wide variation is in part due to differences in dietary formulation (particularly changes in fiber content) (Gidenne, 1987) as well as in computational methodology, which makes TMRT comparisons

difficult. A number of other investigations in the rabbit have involved fiber passage through only part of the GI tract (Laplace et al, 1975; Leng et al, 1977; Uden et al, 1982; Ehrlein et al, 1983; Bjornhag, 1987). GITT for rabbits computed by Sakaguchi and coworkers varied between 11.9 and 84.0 h for different diets (Sakaguchi et al, 1987; Sakaguchi and Hume, 1990), compared to 16.6 h for uninfected rabbits in the present study.

The marker technique, combined with the long post-dose sampling period used in the present study, allowed determination of liquid passage parameters. Liquid TT has been reported as 6.0 (Pickard and Stevens, 1972) and 9.0 (Sakaguchi and Hume, 1990), in agreement with 5.1 h observed in the present study. TMRT was 53.0 h without oral SQXN treatment and 70.8 h with SQXN treatment, while GITT was 57.0 h. Neither parameter has been previously determined for the fluid component of rabbit ingesta. Studying the passage of fine particulates (of less than 75 microns), Sakaguchi and Hume (1990) found TMRT to be 49.2 and 196.5 h, and GITT to be 40.2 and 190.0 h, with considerable inter-individual variation. Their data, for 2 very different diets, demonstrated that fine particulates move with the liquid component of the ingesta and covered the range of values reported in the present study for liquids rather than fiber. Other studies of fluid passage in the rabbit have involved only part of the GI tract (Laplace et al, 1974; Leng et al, 1977; Uden et al, 1982; Gidenne et al, 1988).

GI passage rate parameters vary widely among rabbits on different diets (Brandt and Thacker, 1958; Sakaguchi and Hume, 1990). While certain low-fiber diets appear to improve digestibility by prolonging retention times (Sakaguchi and Hume, 1990), the optimal long-term health of rabbits requires the inclusion of minimal amounts of large fibers in the diet (NRC, 1977). Rather than improving long-term digestibility, reduction in dietary fiber is often associated with cecal hypomotility, delayed mucosal renewal within the GI tract, and altered GI floral balances, which may be associated with common enteritides (NRC, 1977; Cheeke, 1987; Toofanian and Targowski, 1983).

Oral SQXN treatment for 10 to 15 days, at levels normally administered to control coccidiosis within rabbit colonies, had no clinical effects (beyond eliminating oocyst production), but significantly prolonged the TMRT of the liquid component. Alterations in the cecal microflora population, as might be anticipated with oral antibiotic usage, might have resulted in mild cecal distension and hypomotility that could have had this effect. Gastric emptying and small intestinal passage, as well as fecal marker appearance (additionally delayed by cecal distension), are strikingly

prolonged in gnotobiotic rats when compared to conventional rats with normal GI flora, indicating that propulsion along the entire tract is subject to microbial influence (Abrams and Bishop, 1967). Previous investigations of oral antibiotics use in rabbits found none of the growth and digestibility advantages observed with antibiotic usage in swine (Huang et al, 1954). Antibiotic-related prolongation of fiber passage has been demonstrated for swine on certain high-fiber diets, but fluid passage was not investigated (Ravindran et al, 1984).

Marker excretion curves presented by previous investigators demonstrate the diurnal cyclicity of both fluid and fiber component marker concentrations, which was also observed in the present study (Jilge, 1974; Uden et al, 1982; Sakaguchi et al, 1987). This cyclicity, together with the very long TMRT and GITT of the liquid component and the consistently higher concentration of the liquid marker in cecoliths as compared to fecal pellets, substantiates preferential cecotrophic recycling of the fluid component (Bjornhag, 1972; Sakakguchi and Hume, 1990).

Although the single-dose radioisotopic marker technique proved a useful tool for measuring the GI passage rate parameters of rabbits, it had several disadvantages. TT, the most variable parameter in the present study, has similarly been the most variable parameter in ruminant GI studies using this technique (Holleman and White, 1989). However, substantial variability was also present in TMRT and GITT values (as large intra-group and intra-individual CV's), perhaps because the assumption of unchanged intake and constant outflow was unrealistic for the experimental period.

Another basic assumption of the technique is that marker excretion will be a relatively continuous process (Holleman and White, 1989). While ruminants excrete feces frequently, rabbits normally excrete no fecal pellets for more than 50% of each 24-hour period. This is in part due to the rabbits unique abilities in rapidly clearing bulky fibrous material from the tract (Bjornhag, 1987) and to the relatively long periods of cecotrophic reingestion. As a result, it is impossible to obtain or even approach continuous marker excretion by sampling frequently at evenly spaced intervals. For other herbivores, the fecal marker excretion curve can be accurately characterized by collecting numerous regular samples in the initial post-dosing period and fewer widely-spaced samples later.

Some investigators have attempted to circumvent some of the difficulties of marker excretion sampling in rabbits by using Elizabethan collars, thereby collecting cecoliths (Piekarz, 1963; Jilge, 1974; Leng et al, 1977; Pickard and Stevens, 1982; Uden et al, 1982; Ehrlein et al, 1983). This

method was deemed unacceptable in the present study because of the potential for disruption of normal GI physiology observed during the first RI trial. However, nearly all rabbits spontaneously dropped cecoliths during these RI studies and cecoliths formed a relatively consistent percentage of the total excreted samples for all tested rabbits, so that marker concentrations in these fecal forms were available to compute the linear regression lines modeling excretion. It may be validly argued that inclusion of cecolithic marker concentrations tends to bias the results of TMRT and GITT data toward longer residence times, particularly for the liquid component. However, use of this data allowed a reasonable estimate of the very prolonged fluid passage parameters in rabbits which have previously been unobtainable (Pickard and Stevens, 1972; Uden et al, 1982; Sakaguchi et al, 1987; Sakaguchi and Hume, 1990).

The potential errors introduced by including cecoliths were felt to be small relative to the large inter-individual variations observed for rabbit GI passage rate parameters in this and previous studies (Uden et al, 1982; Sakaguchi et al, 1987; Sakaguchi and Hume, 1990). Large intra-individual differences were also observed for those few rabbits tested repeatedly at the same infection status. Study design would have been improved by repeated measuring of all individuals prior to infection and at regular intervals post-dosing, so that each individual could act as its own control. The small scale of the present study and the complications introduced by the low productivity of the Obeliscoides isolate (Nielsen, Chap. 1) precluded this option.

Observation of substantial inter-individual variations in GI passage rate parameters in the present study suggest the operational efficiency of the colonic separation mechanism (Bjornhag, 1972, 1987) may vary even among rabbits on the same diet. As an example, in the present study cecoliths happened to be available from 2 uninfected rabbits (of nearly identical age and body weight, given similar marker doses) at 68 and 69 hours after marker administration. For these 2 cecolith samples, specific activity of the fiber marker was 313 and 3 cpm/g dried sample, respectively (liquid marker was 251 and 337 cpm/g), and fiber marker concentrations fell below detectability at 164 and 170 hours. Such a large difference in the concentration of fiber marker present in cecoliths at the same time post-dosing, despite nearly identical systemic elimination times, is consistent with substantial individual differences in the operational efficiency of the colonic separation mechanism. It seems possible that rabbits may differ substantially in the percentage of large fibers allowed to enter the cecum and mix with its contents, and thus in the rate at which fibers are retained (as reingested cecoliths) or eliminated from the system (as fecal pellets). Such an efficiency variation could explain

the large individual differences in fiber digestibility previously proposed for rabbits (Sakaguchi et al, 1987; Sakaguchi and Hume, 1990).

GI passage rate parameters in Obeliscoides-infected rabbits

A primary goal of the present study was to determine the rate-constant of pyloric exit prior to and following trichostrongylid infection, and to detect any effects such infections might have on gastric emptying. Because the stomach is relatively voluminous and because of the rapid, unimpeded exit of large fibers (>315 microns, Bjornhag, 1972) from the rabbit GI tract beyond the pylorus, it is reasonable that marker dilution within the stomach might represent the rate-limiting relative turnover as expressed by the parameter GITT. This is why Sakaguchi et al (1987) proposed that the rate constant "k" (reciprocal of GITT) of fiber marker excretion would reflect this marker's gastric dilution. However, this estimate is accurate only if the fiber marker largely avoids entering the cecum, and is therefore dependent on the high-efficiency operation of the colonic separation mechanism. In the present study, as noted, this efficiency appeared to vary considerably between individuals.

Utilization of the single-dose dual RI marker technique presented 2 difficulties in determining the effects of Obeliscoides infection on GI passage. (1) When GI passage rate parameters were evaluated for infected rabbits at various times post-inoculation, relatively broad variations were encountered within these groupings. This was not unexpected, since inter- and intra-individual differences had been noted in evaluating the passage rate parameters of uninfected rabbits. (2) Anorexia during the first 3 post-inoculation weeks was the primary clinical sign of Obeliscoides infection observed in the present study. While there was some evidence that anorexia tended to prolong all passage rate parameters for both fiber and liquid components (Table 4.10), the single-dose marker technique requires the assumption that food intake and fecal output remained unchanged over the excretion period. Because a 10-day excretion period was necessary to obtain substantial marker recovery, and because anorexia and functional GI disruptions often lead to cessation of fecal output in rabbits (Cheeke, 1987), RI passage studies were necessarily delayed until anorexia had passed and modest fecal output was observed.

None of the liquid passage rate parameters and in most of the fiber passage rate parameters observed in infected rabbits were significantly different from those of uninfected controls. In particular, the rate of gastric dilution of the fiber marker, GITT, considered an indicator of effects

on pyloric outflow (Sakaguchi et al, 1987), was not significantly affected by primary Obeliscoides infection during PIW 8-9, despite significant increases in serum gastrin concentration during PIW 6 and 7 in this group (Nielsen, Chap. 3).

Both TMRT and GITT of the fiber component were significantly prolonged during PIW 16 to 26 for rabbits with secondary Obeliscoides infections. These observations were of 2 clinically normal rabbits (not treated with SQXN) at the end of long (13- and 15-wk), productive patencies. Theirs were the only patent secondary infections observed in this study. Fecal excretion of N, P or Ca remained unchanged during an overlapping interval (PIW 16-34). While neither serum nor necropsy information was available from rabbits with secondary infections during PIW 16 to 26, evidence from an earlier interval (PIW 10-15) indicated enhanced Ca absorption from the posterior small intestine (discussed above) and normal serum gastrin concentrations (Nielsen, Chap. 3).

Chronic alterations in GI passage rate parameters have been suggested to cause the reductions in food intake and nutrient digestibility associated with both rabbit Obeliscoides infections (Pace and Frandsen, 1982) and ruminant Ostertagia spp. (Sykes, 1977). The common and often persisting effects of trichostrongylids on gastric pH, serum gastrin, and GI myoelectric activity all suggest that ingesta passage may be chronically affected (Bueno and Fioramonti, 1980; Bueno, Dakkak and Fioramonti, 1982; Titchen 1982; Dakkak, 1984; Holmes, 1985; Symons, 1989). The recent work of Fox and coworkers (Fox, Gerrelli, Pitt, Jacobs, Gill and Gale, 1989) finally demonstrated that during PIW 6-7 (counting from the time of the first of repeated larval inoculations), ingesta passage was prolonged in Ost. ostertagi-infected calves. Reduced passage rate persisted even after anthelmintic treatment during PIW 9-10. Passage rate reductions were also observed in uninfected, pair-fed control calves, suggesting that motility effects may be inextricably related to intake reduction. However, passage rate prolongation, anorexia, and increased pH were all temporally related to a peak in serum gastrin concentrations observed in infected calves during PIW 5-6 (Fox, Gerrelli, Pitt, Jacobs, Gill and Simmonds, 1989).

In contrast to these observations on infected ruminants, Obeliscoides infection of rabbits in the present study was associated with only limited effects on ingesta passage, which were not temporally related to changes in either serum gastrin or to apparent nutrient absorption. Larger-scale studies using the dual RI marker technique, in which each individual rabbit acts as its own control, may allow better evaluation of the effects of Obeliscoides infection on passage rate while avoiding

the physiological disruptions inherent in cannulation or radiological techniques.

Conclusions.

In both primary and secondary Obeliscoides infections, early anorexia and enhanced fecal N excretion occurred within a time-frame similar to that reported for Ostertagia-infected ruminants. Following a period of enhanced fecal N loss, compensatory enhancement of N absorption by the posterior small intestine apparently occurred. Possible effects on the intestinal absorption of Ca absorption were also indicated, and gastrointestinal fiber passage was prolonged at one stage of the infection. These effects on GI function were induced by an Obeliscoides isolate with very low patency and a considerable tendency toward inhibition (Nielsen, Chap. 1). Despite their immature and often stunted appearance, these trichostrongylids persisted in the stomachs of rabbits for as long as 45 weeks post-inoculation and were associated with many of the same deleterious effects on nutrient absorption and flow reported for ruminants with active abomasal trichostrongylids.

The present study tended to validate the impression that chronic, subclinical gastric trichostrongylid infections can affect animal productivity by subtle disruptions of GI function. Larval trichostrongylids in particular may remain subclinical for long periods within the abomasal mucosa, where they are notoriously resistant to many anthelmintic treatments. The continuous maturation of small percentages of the inhibited population over weeks or months post-ingestion can subtly affect intake, motility, pH, serum gastrin, and nutrient utilization (Sykes, 1977; Parkins et al, 1982a,b; Holmes, 1985; Brunsdon et al, 1986; Symons, 1989). Utilization of Obeliscoides-infected rabbits could provide an important laboratory model for examining the mechanisms involved.

A need to understand the pathophysiological causes of economically costly aspects of Ostertagia infections in commercial ruminant hosts is usually cited as the primary reason laboratory models of these infections are important. However, many wildlife species harbor gastric trichostrongylids, including the Alaskan snowshoe hares which were the source of the present isolate. Wild herbivores are subjected to seasonal nutrient deprivation (Pehrson, 1980, 1983), but because confinement of wildlife introduces problems of dietary and habitat artificiality as well as stress, the pathogenesis of Obeliscoides infections in hares and of Ostertagia spp. infections in wild ruminants have not been studied in living natural hosts. A laboratory model elucidating the physiological effects of such parasitism could be an important key to understanding the nutritional ecology of hares and

other wild species.

SYNOPSIS AND CONCLUSIONS

Obeliscoides sp. was isolated from Alaskan snowshoe hares in the northwestern extreme of their North American range and passaged for 3 generations in laboratory rabbits. The biological features of this isolate and the pathogenic effects it had on the stomach, its in-host habitat, are presented in Chapter 1. Although its prepatent period and length of patency were similar to those of previous isolates, the present strain was characterized by its low fertility, its ability to cause chronic gastric lesions, and its persistence as occult (nonpatent) infections in clinically normal rabbits. Immature, stunted and infertile Obeliscoides were recovered from the gastric mucosa as long as 45 weeks after oral inoculation. The tendency to arrest maturation appeared to be an inherent feature of the Alaskan isolate, suggesting it is one of the long-lived strains favored in some cyclic host populations (Shaw and Moss, 1989).

The most severe gastric lesions were observed in previously uninfected rabbits (primary infections) that were necropsied within 15 weeks of larval inoculation. Epithelial hyperplasia increased mucosal thickness and was accompanied by an intense eosinophilic infiltration in the fundic zone. In rabbits which had been previously infected (secondary infections), fundic lesions tended to be erosive rather than hyperplastic and were more localized. Nodular hyperplasia occurred in the pyloric zone of nearly all infected rabbits. Dense local mononuclear aggregations were observed in the deep mucosa of primary infections older than 15 weeks and secondary infections older than 9 weeks. These follicular structures were morphologically similar to those characteristic of pre-type II Ostertagia infections in ruminants. Both the tendency to arrest maturation and the characteristics of these lesions suggest that the present isolate may be a useful model for understanding pre-type II ostertagiasis (Snider et al, 1981, 1983, 1985).

The total number of Obeliscoides present in the rabbit host's stomach at the time of necropsy directly affected the severity of gastric lesions, even though many of the parasites appeared immature, stunted or infertile. Number of trichostrongylids was in turn directly related to the age of the infection. Therefore, time post-inoculation (i.e. post-inoculationweek, PIW) was the independent variable used to analyze the pathophysiological effects of these infections. Primary and secondary infections were evaluated separately. Obeliscoides populations in secondary infections were generally

smaller and decreased more quickly, and the nature of the lesions was different from those in primary infections. Pathophysiological effects of Obeliscoides infections included an early anorectic period associated with changes in serum protein parameters suggesting reduced protein absorption (Chapter 2). Consistent with that observation, fecal N excretion increased in the early weeks of both primary and secondary infections (Chapter 4). Compensatorily enhanced N absorption (Chapter 4), hypergastrinemia (Chapter 3), hypermagnesemia and hypokalemia (Chapter 2), and prolongation of passage rate for the fiber component of the ingesta (Chapter 4) were transiently observed during later weeks when infected rabbits were clinically normal.

Of the 31 Obeliscoides infections observed (21 primary, 10 secondary), 16 rabbits (12 primary, 4 secondary) manifested clinical signs of anorexia. Excluding 2 dexamethasone-treated rabbits, anorexia was only apparent within 3 weeks after larval inoculation. Anorectic rabbits were characterized by significant reductions in their serum total protein, albumin, and A/G ratios (Table 2.3, Chapter 2) which were not observed in fasted, uninfected rabbits or in infected rabbits which ate normally. However, significant increases in the fecal excretion of N were observed for all Obeliscoides-infected rabbits, including both primary and secondary infections, during PIW 1 and 2 (Table 2.2, Chapter 2). Increased fecal N excretion continued to PIW 5 in rabbits with primary infections (Table 2.2). When necropsied between PIW 5 and 15, however, concentrations of N in the distal small intestine were very low (Table 4.5, Chapter 4), indicating enhanced absorption of this nutrient. While the first 5 weeks of primary Obeliscoides infections were characterized by signs of increased fecal N loss, observations from the following 10 weeks were consistent with a period of augmented intestinal N absorption.

Previous investigations of the nutritional effects of Obeliscoides infections in rabbits have demonstrated reduced N digestibility within the first 3 weeks of infection (Pace and Frandsen, 1982). Reduced protein digestibiliy, low serum protein parameters, and increased nitrogenous loss are important factors accounting for costly production losses in ruminant Ostertagia infections (Sykes and Coop, 1977; Parkins et al, 1982a,b; Holmes, 1985). Compensatory enhancement of N absorption appears to be a major adaptation ruminants can make to Ostertagia parasitism (Steel and Symons, 1982; Sykes, 1987; Symons, 1989). In wild hare populations, a similar adaptation to Obeliscoides parasitism might be vital for survival and reproduction given the pronounced seasonality of nutrient availability.

Rabbits with both patent and occult infections maintained normal ration intake and cecotrophy beyond 3 weeks after larval inoculation, yet alterations in the concentrations of several serum constituents occurred between PIW 5 and 15. Significant hypokalemia was apparent in rabbits with primary infections during PIW 5 (Table 2.2, Chapter 2), perhaps associated with enhanced GI mucus production. Hypermagnesemia occurred in both primary and secondary infections between PIW 8 and 15 (Table 2.2, Chapter 2), but the hypocalcemia and hypophospatemia reported in some ruminant trichostrongylid infections (Coop et al, 1977, 1981; Sykes et al, 1988) was not observed.

Transient increases in serum Mg might reflect alterations of the GI lumenal balance among Mg, Ca and IP (inorganic phosphorus). There was evidence for enhanced Ca absorption in some infected rabbits during PIW 10-15, and for increased fecal excretion of Ca during PIW 16-34. Rabbits absorb Ca from the GI tract in a relatively unregulated manner and substantial fluctuations of Ca within the GI lumen are probably common. Therefore, the mineral absorption and serum concentration responses of this host species to trichostrongylid-induced pH changes may differ substantially from those of ruminants. Increased serum magnesium concentrations also might have been facilitated by enhanced absorption of this mineral through reductions in its ionization in the anterior intestine that would accompany increased gastric alkalinity.

Because increased gastric alkalinity and increased serum concentrations of the acid-stimulating peptide hormone gastrin are common features of ruminant Ostertagia infections, RIA techniques were used for the first time to detect rabbits' serum gastrin responses to Obeliscoides infection. In normal rabbits, gastrin concentrations averaged 65 pg/ml, and significant increases (to 176 and 177 pg/ml) were detected in infected rabbits during PIW 6 and 7 (Table 3.1, Chapter 3). While gastric pH at necropsy was unchanged by infection (Tables 1.5 and 1.6, Chapter 1), this value was measured for only 2 rabbits (euthanized during PIW 5) prior to PIW 10.

Hypergastrinemia, hypokalemia and hypermagnesemia temporally coincided with the early patent period between PIW 5 and 10 in rabbits experiencing primary Obeliscoides infections. In rabbits with secondary infections, most of which were not patent, hypergastrinemia and hypermagnesemia were still observed, between PIW 7 and 15. Obeliscoides patency began and increased between 5 and 15 weeks after larval inoculation in many of the infected rabbits, but these serum alterations were independent of parasite egg production in individual hosts.

Since gastrin affects GI tract motility, increased serum gastrin concentrations indicates potential alterations in the rate of passage of ingesta, with potential collateral effects on appetite, absorption and serum concentrations of minerals and other substances, and fecal nutrient excretion. Ingesta passage rate measurement in the rabbit is complicated by preferential recycling of fluids and small particulates via cecotrophy. The use of a single-dose radioisotopic marker technique allowed the first determination of fluid retention times in rabbits: TMRT was 53.0 h and GITT was 57.0 h (Table 4.9, Chapter 4). Obeliscoides infection did not significantly affect fluid passage rate parameters (Table 4.9). The use of standard doses of sulfaquinoxaline to control coccidiosis, however, significantly prolonged TMRT, to 70.75 (Table 4.9), possibly via effects on the cecal flora.

Passage rates of the fiber component of the ingesta were very rapid compared to those for fluid, with TMRT of 19.9 h and GITT of 16.6 h (Table 4.8, Chapter 4). Limitation of the marker technique precluded evaluation of passage rates for anorectic rabbits. The potential of fiber passage rate parameters to measure the rate of pyloric emptying in trichostrongylid-infected rabbits was limited by large individual variations in these parameters, and apparently large differences in efficiency of operation of the colonic separation mechanism. Nevertheless, fiber passage was significantly prolonged in rabbits with secondary Obeliscoides infections during PIW 16 to 26 (TMRT of 31.8 h and GITT of 26.3 h, Table 4.8). The affected rabbits were clinically normal and harbored small, senescent trichostrongylid populations.

The capacity of the Alaskan strain of Obeliscoides to significantly affect physiological parameters of clinically normal hosts was surprising. In many cases, small populations of parasites that appeared to be immature or stunted, undetectable in the live host because of their repoductive inactivity, produced significant mucosal lesions and exerted detectable effects on host physiology over a prolonged period. Since this type of infection has often been ignored in studies of wild Lepus populations, the potential impact of occult trichostrongylid infections on the nutritional ecology of their natural hosts should be reconsidered. Because many of the physiological effects are similar to those reported for Ostertagia-infected ruminants, rabbits infected with this strain of Obeliscoides may prove an economical laboratory model system for understanding the complex basis of productivity losses in both commercial ruminants and wild herbivores.

APPENDIX A: PROTOCOL OF 8 OBELISCOIDES EXPERIMENTS (in 12 subdivisions) IN RABBITS

1.	Experiment			
	a. No., subdivision	1	2a	2b
	b. inoculation no.	1-3	4 & 5	6 & 7
	c. duration (wks)	2-5	17 & 23	9 & 14
2.	Number of rabbits infected			
	a. water (control)	2	0	0
	b. primary infections	3	2	0
	c. secondary infections	0	0	2
3.	Host age (wks)			
	a. at start	18-19	23	23
	b. at end	22-23	35 & 45	32 & 35
4.	Host weight (kg)			
	a. at start	3.5-3.6	3.3 & 3.5	3.5
	b. at end	2.8-3.5	4.0	3.7 & 4.2
5.	Infective Obeliscoides larvae (L3) used			
	a. isolate/passage*	La3798	La3806	La3807
	b. primary dose size**	500-2500	2500	-
	c. secondary dose size**	-	-	2600
	d. refrig. time (wks)	1/2-2	none	none
	c. inoculation method:	tube	capsule	capsule
6.	Sulfaquinoxaline, PO			
	a. no. treated	none	none	none
	b. tmt. period PID (PIW)			
7.	Dexamethasone, IM			
	a. no. treated	none	1	1
	b. days pre-inoculation		none	none
	c. post-inoc. PID (PIW)		89-118 (13-17)	84-99 (12-15)
8.	Parturition, lactation			
	a. no. affected	none	1	1
	b. lactation PID(PIW)***		115-180 (17-26)	50-62 (8-9)

Appendix, page 2

1.	Experiment			
	a. No., subdivision	3a	3b	4a
	b. inoculation no.	8-11	12	13 & 14
	c. duration (wks)	8-44	8	7-13
2.	Number of rabbits infected			
	a. water(control)	0	0	0
	b. primary infections	4	1	0
	c. secondary infections	0	0	2
3.	Host age (wks)			
	a. at start	10	10	18
	b. at end	18-54	18	25-31
4.	Host weight (kg)			
	a. at start	1.8-2.4	2.4	3.0-3.5
	b. at end	3.0-4.5	3.3	3.3-3.7
5.	Infective Obeliscoides larvae (L3) used			
	a. isolate/passage*	La3798,La3806, La3807	FP (3806-88)	FP (3806-37)
	b. primary dose size**	1700-15,000	2800	-
	c. secondary dose size**	-	-	8300-13000
	d. refrig. time (wks)	8-16	3	none to 3
	e. inoculation method	capsule	capsule	capsule
6.	Sulfaquinoxaline, PO			
	a. no. treated	none	none	none
	b. tmt. period PID (PIW)			
7.	Dexamethasone, IM			
	a. no. treated	none	none	2
	b. days pre-inoculation			7
	c. post-inoc. PID (PIW)			0-21 (1-3)
8.	Parturition, lactation			
	a. no. affected	none	none	none
	b. lactation PID(PIW)***			

Appendix, page 3

1.	Experiment			
	a. No., subdivision	4b	5	6
	b. inoculation no.	15	16 & 17	18-22
	c. duration (wks)	13	39 & 45	2-41
2.	Number of rabbits infected			
	a. water (controls)	0	0	0
	b. primary infections	0	0	5
	c. secondary infections	1	2	0
3.	Host age (wks)			
	a. at start	18	26 & 45	10
	b. at end	31	65 & 91	12-47
4.	Host weight (kg)			
	a. at start	3.5	4.0 & 4.1	1.8-2.0
	b. at end	3.7	4.7 & 5.2	1.4-4.3
5.	Infective Obeliscoides larvae (L3) used			
	a. isolate/passage*	FP (3807-37)	FP (3807-37)	FP (3807-37)
	b. primary dose size**	-	-	16,300-36,000
	c. secondary dose size**	19,600	45,500&50,400	-
	d. refrig. time (wks)	(1 day)	none to 6	1/2-8
	e. inoculation method:	capsule	tube	tube
6.	Sulfaquinoxaline, PO			
	a. no. treated	none	2	2
	b. tmt. period PID (PIW)		243-258 (35-37)	243-258 (35-37)
7.	Dexamethasone, IM			
	a. no. treated	none	none	none
	b. days pre-inoculation			
	c. post-inoc. PID (PIW)			
8.	Parturition, lactation			
	a. no. affected	none	none	none
	b. lactation PID(PIW)***			

Appendix, page 4

1.	Experiment			
	a. No., subdivision	7a	7b	7c
	b. inoculation no.	23-25	29 & 30	26-28
	c. duration (wks)	11-15	5 & 11	10-13
2.	Number of rabbits infected			
	a. water (control)	0	0	0
	b. primary infections	3	2	0
	c. secondary infections	0	0	3
3.	Host age (wks)			
	a. at start	21	27	38 & 54
	b. at end	31-35	32 & 37	47,48,67
4.	Host weight (kg)			
	a. at start	3.2-3.6	3.5 & 4.0	3.9-4.5
	b. at end	3.7-4.1	3.8 & 4.3	4.2-4.9
5.	Infective Obeliscoides larvae (L3) used			
	a. isolate/passage*	SP	SP	SP
	b. primary dose size**	29,700-31,400	29,500&30,900	-
	c. secondary dose size**	-	-	41,300-44,300
	d. refrig. time (wks)	9-22	15-28	7-22
	c. inoculation method	tube	tube	tube
6.	Sulfaquinoxaline, PO			
	a. no. treated	3 (twice)	2	3 (twice)
	b. tmt. period PID (PIW)	9-24 (2-4)& 51-66 (8-10)	8-23 (2-4)	9-24 (2-4) & 51-66 (8-10)
7.	Dexamethasone, IM			
	a. no. treated	none	none	none
	b. days pre-inoculation			
	c. post-inoc. PID (PIW)			
8.	Parturition, lactation			
	a. no. affected	none	none	none
	b. lactation PID(PIW)***			

Appendix A, page 5

1.	Experiment		
	a. No., subdivision	8	8 Expt's, as 12 subdivisons
	b. inoculation no.	31	31 larval inoculations
	c. duration (wks)	5	2 to 45 weeks
2.	Number of rabbits infected		
	a. water (control)	1	3 controls (water)
	b. primary infections	1	21 primary infections
	c. secondary infections	0	10 secondary infections
3.	Host age (wks)		
	a. at start	23	10 to 45 weeks
	b. at end	28	12 to 91 weeks
4.	Host weight (kg)		
	a. at start	4.0	1.8 to 4.5 kg
	b. at end	4.1	1.4 to 5.2 kg
5.	Infective Obeliscoides larvae (L3) used		
	a. isolate/passage*	TP	3 La, 2 FP, 1 SP, 1 TP
	b. primary dose size**	45,500	500 to 45,500 larvae
	c. secondary dose size**	-	2600 to 50,400 larvae
	d. refrig. time (wks)	1/2-2	none to 28 weeks
	c. inoculation method:	tube	tube=7 subdiv's, capsule=6 subdiv's
6.	Sulfaquinoxaline, PO		
	a. no. treated	1	13 rabbits in 6 subdiv's
	b. tmt. period PID (PIW)	16-26 (3-4)	
7.	Dexamethasone, IM		
	a. no. treated	none	4 rabbits in 2 subdiv's
	b. days pre-inoculation		
	c. post-inoc. PID (PIW)		
8.	Parturition, lactation		
	a. no. affected	none	2 rabbits in 2 subdiv's
	b. lactation PID(PIW)***		

Appendix A: footnotes

* Isolates/passage: infective larvae derived from
La=Lepus americanus (3 individuals)
FP=first passage infection (2 rabbits)
SP=second passage infection, combined from 4 rabbits
TP=third passage infection (1 rabbit)

** Dose size = number of infective larvae (L3) in dose
*** Parturition is counted as "lactation day 1"

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